The Role of Parental Care in Shaping Personality-Related Variation in Reproductive Success in Free-living Passerines

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Summary

Animals from a wide range of taxa show repeatable individual differences in behaviour. Moreover, behavioural traits often covary with proxies for fitness, such as survival and reproductive success, implying that they are under natural selection. Yet, we still know very little about why these differences exit. To acquire insight into the maintenance, functional significance and evolution of such consistent individual differences in behaviour, we need to investigate the mechanisms maintaining behavioural variation and also the specific proximate and ultimate pathways mediating links between behaviour and fitness.

Selective mechanisms maintaining behavioural variation include various forms of heterogeneous selection, e.g. temporal fluctuation in selection pressures and sexually antagonistic selection, and other types of selection, e.g. correlational selection and life-history trade-offs. Yet, these mechanisms can only explain why individuals differ in behaviour, but not why these differences should be consistent across time or contexts. Theoretical models for explaining consistent between-individual differences in behaviour are mostly based on the assumption that individuals differ in state and that behaviours are state-dependent. Behaviours that depend on the state of an individual are thus expected to be consistent when the underlying state variable, e.g. body size, is inherently stable. Yet, even labile states, e.g. amount of energy resources or levels of hormones, can explain consistency in behaviour when positive feedback loops between behaviour and state stabilize and strengthen initial small differences in state.

Thus, to fully understand why animals consistently differ in their behaviour from an adaptive point of view, it is also important to identify the proximate state variables meditating this variation. The androgen hormone testosterone is well known to affect behaviours such as aggressiveness and parental care, and is therefore a promising candidate for underpinning behavioural variation.

Furthermore, understanding how natural selection acts on behaviour requires identifying mechanistic pathways linking behaviour with fitness. One variable that is likely to play an important role in mediating links between personality and reproductive success is parental care. This is because parental care directly affects reproductive output, while levels of parental care are expected to differ between behavioural phenotypes. The probably most intensively studied aspects of parental care are offspring provisioning behaviour and defense of nestlings against predators.

Our insight into the mechanisms linking personality with reproductive success might be hampered by merely using observational approaches. This is because potential links between personality, parental care and reproductive success might be obscured by behavioural phenotypes differing in other aspects that simultaneously affect brood size and parental care, e.g. habitat quality. This could result in behavioural types consistently experiencing a different environment. Investigating whether personality types differ in parental behaviour per se, therefore requires breaking apart such potential personality-environment correlations. In chapter 4, we did this by experimentally determining the brood size that each individual were given to raise (reduced, control or enlarged).

In chapter 1, I provide observational and experimental evidence that individual differences in circulating plasma testosterone levels do not cause variation in exploratory behaviour in male house sparrows. This finding suggests that this androgen hormone is most likely not part of an underlying proximate mechanisms causing and maintaining individual variation in exploratory behaviour. Yet,

between-individual differences in this state variable could still be part of a physiological mechanisms underpinning variation in other behavioural traits, e.g. aggressiveness and parental care.

Chapters 2, 3 and 4 show that parental care is involved in mediating the link between personality and fitness. I found strong evidence for parental provisioning behaviour playing a key role in linking personality with reproductive success in blue tits (chapter 3). Aggressive males and slow-exploring females fed their nestlings at the lowest rates. While the relative low investment in offspring provisioning of slow-exploring females also hampered their reproductive success, aggressive males produced more fledglings despite their low feeding rate. This was because their low feeding rates were associated with an increased effort of their female partners. Since parental care is costly, this raises the question of why females mated with aggressive males are willing to take a larger share of the burden of care. Possibly, aggressive males provide their mates with other benefits, such as high-quality territories or good genes, or invest more in other aspects of parental care, such as defense of offspring against nest predators.

We indeed found support for the notion that pairs of blue tits divide different parental care duties among partners, with one pair member investing more in nest defense, while the other continued offspring provisioning when confronted with a nest predator (chapter 2). Such a division of labour could help to ensure immediate nestling survival while at the same time mitigating potential negative long-term consequences of feeding interruptions for offspring growth and condition, thus maximizing fitness. Possibly, aggressive males take a larger share of defending the brood against potential nest predators, while their mates, in return, put more effort into offspring provisioning.

Chapter 4 presents evidence for selection acting on exploratory behaviour in great tits, with selection pressures differing across years and sexes. In females,

fast-exploratory tendency was selected for in both years, but pathways mediating the link between personality and reproductive success differed between years. In a 'good' year, where food availability was high, fast exploring females had higher brood sizes prior to manipulation, but did not feed their manipulated broods at higher rates (compared to slow explorers with similar manipulated broods sizes), resulting in a higher number of fledglings that were in worse condition. This suggests that dissimilar exploration types resolved the trade-off between fledgling number and condition in different ways. In contrast, in a 'bad' year, fast-exploring females generally did better with producing more and heavier fledglings. This implies that fast-exploring females coped relatively well with the adverse environmental conditions of this year. Yet, this link was not mediated by differences in initial clutch size or provisioning behaviour, suggesting that other mechanisms (not explicitly considered in our study) were involved in mediating links between behaviour and fitness. For instance, fast-exploring females might increase their reproductive success by investing more in nest defense behaviour or providing further forms of parental care (e.g. brooding of nestlings).

In male great tits, exploratory tendency was only selected for in a 'bad' year and differed between brood size manipulation categories. While slow explorers produced more and heavier fledglings when raising natural brood sizes, they had lighter fledglings compared to fast explorers when their brood size was experimentally reduced. These links were not mediated by personality-related provisioning behaviour. Yet, we found some evidence for slow explorers bringing a higher proportion of high quality prey items, i.e. caterpillars, which could explain their enhanced reproductive success. The finding that selection patterns on exploratory behaviour differ between sexes is consistent with previous studies on great tits and now warrants further attention.

In summary, my work provides evidence that parental care plays an important role in mediating links between personality and fitness. Yet, these pathways do not seem

to be ubiquitous, but instead differ between species, years and sexes. My thesis thus helps to shed light on our understanding of how natural selection can shape behaviour, and how it can maintain behavioural variation. My studies additionally suggests that other mechanisms, not yet considered, are involved in mediating behaviour-fitness relationships, emphasizing the importance of continued studies in this field.

Zusammenfassung

Tiere aus einer großen Anzahl an Taxa zeigen stabile individuelle Verhaltensunterschiede. Darüber hinaus kovarieren Verhaltenzüge oftmals mit Fitnessvariablen, wie Überlebenswahrscheinlichkeit und Fortpflanzungserfolg. Dies impliziert, dass das Verhalten von Tieren unter natürlicher Selektion steht. Wir wissen jedoch nach wie vor sehr wenig darüber warum diese Unterschiede existieren. Um Einblick in den Erhalt, die funktionelle Bedeutung und Evolution von solchen stabilen individuellen Verhaltensunterschieden zu bekommen, müssen wir zum einen die Mechanismen untersuchen, die diese Verhaltensvariation erhalten und zum anderen die proximaten und ultimaten Pfade identifizieren, die Verhaltensweisen mit Fitness verknüpfen.

Selektionsmechanismen, die Verhaltensvariation erhalten können, umfassen heterogene Selektionsformen, wie z.B. zeitliche Schwankung in Selektionsdrücken und sexuelle antagonistische Selektion, und andere Arten von Selektion, wie z.B. korrelative Selektion und Life-history Trade-offs. Diese Selektionsformen können erklären warum Individuen sich in ihrem Verhalten unterscheiden, jedoch nicht warum diese Unterschiede über längere Zeiträume und über verschiedene Kontexte hinweg stabil seien sollten. Theoretische Modelle für die Erklärung von stabilen zwischen-individuellen Unterschieden basieren hauptsächlich auf der Annahme, dass sich Individuen in ihrem Zustand („state“) unterscheiden und dass Verhalten gleichzeitig zustandsabhängig ist. Verhaltensweisen, die von dem Zustand eines Individuums abhängen, sollten demzufolge stabil sein, wenn der dem Verhalten zu Grunde liegende Zustand, z.B. Körpergröße, an sich stabil ist. Aber sogar labile Zustände, wie z.B. die Höhe der Energiereserven oder Hormonspiegel, können

die Stabilität von Verhalten erklären, wenn positive Rückkopplungsmechanismen zwischen Verhalten und Zustand die ursprünglich geringen Unterschiede im Zustand stabilisieren und verstärken.

Um von einer adaptiven Perspektive aus zu verstehen, warum sich Tiere beständig in ihrem Verhalten unterscheiden, ist es deswegen wichtig die proximaten Zustandsvariablen zu identifizieren, die diese Variation vermitteln. Das Androgenhormon Testosterone ist dafür bekannt Verhaltensweisen, wie Aggressivität und elterliche Fürsorge, zu beeinflussen und ist folglich ein vielversprechender Kandidat um Verhaltensvariation zu unterstützen.

Um zu verstehen wie natürliche Selektion sich auf Verhalten auswirkt ist es notwendig mechanistische Pfade zu identifizieren, die Verhalten mit Fitness verknüpfen. Eine Variable, die aller Wahrscheinlichkeit nach eine wichtige Rolle dabei spielt Persönlichkeit mit Reproduktionserfolg zu verkoppeln, ist elterliche Fürsorge. Der Grund dafür ist, dass elterliche Fürsorge den Reproduktionserfolg direkt beeinflusst, während sich gleichzeitig der Level von elterliche Fürsorge zwischen Verhaltensphenotypen unterscheiden sollte. Die wahrscheinlich am Besten untersuchten Aspekte von elterlicher Fürsorge sind Fütterverhalten und Verteidigung von Nestlingen gegen Räuber.

Unser Einblick in die Mechanismen, die Persönlichkeit mit Fortpflanzungserfolg verknüpfen, wird durch die ausschliessliche Verwendung von auf Beobachtungen basierten Studien beeinträchtigt. Der Grund dafür ist, dass potentielle Verknüpfungen zwischen Persönlichkeit, elterlicher Fürsorge und Fortpflanzungserfolg dadurch verschleiert werden könnten, dass sich Verhaltenstypen in anderen Aspekten unterscheiden, die sowohl Brutgröße als auch elterliche Fürsorge beeinflussen, wie z.B Habitatqualität. Dies könnte zur Folge haben, dass Verhaltenstypen beständig unterschiedlichen Umwelten ausgesetzt sind. Um zu untersuchen, ob Persönlichkeitstypen sich *per se* in ihrem elterlichen Verhalten unterscheiden,

ist es notwendig solche potentiellen Persönlichkeits-Umwelt-Korrelelationen aufzubrechen. In Kapitel 4, lösten wir diese Verknüpfung in dem wir die Brutgröße, die ein Individuum aufziehen musste, experimentell festlegten (verkleinert, gleichbleibend, vergrößert).

In Kapitel 1, liefere ich auf Beobachtungen basierende und experimentelle Hinweise dafür, dass Variation im Erkundungsverhalten in männlichen Hausspatzen nicht durch individuelle Unterschiede in Plasma-Testosteronspiegeln verursacht werden. Diese Erkenntnis legt nahe, dass dieses Androgenhormon nicht Teil eines grundlegenden proximaten Mechanismus ist, der individuelle Variation in Erkundungsverhalten verursacht und erhält. Zwischen-individuelle Unterschiede in dieser Zustandsvariablen könnten dennoch Teil eines physiologischen Mechanismus sein, der Variation in anderen Verhaltenszügen, z.B. Aggressivität und elterliche Fürsorge, unterstützt.

Kapitel 2, 3 und 4 zeigen dass elterliche Fürsorge daran beteiligt ist Persönlichkeit und Fitness zu verknüpfen. Ich liefere wichtige Hinweise dafür, dass elterliches Fütterverhalten in Blaumeisen eine Schlüsselrolle bei der Verlinkung von Persönlichkeit und Fitness spielt (Kapitel 3). Aggressive Männchen und langsam erkundende Weibchen fütterten ihre Jungen am Seltensten. Während das relativ geringe Investment in das Füttern des Nachwuchses bei langsam erkundenden Weibchen ihren Fortpflanzungserfolg beeinträchtigte, produzierten aggressive Männchen trotz ihrer niedrigen Fütterrate mehr Nachkommen. Dies kam dadurch zustande, dass die niedrigen Fütterraten aggressiver Männchen mit erhöhtem Einsatz ihrer Partnerinnen verbunden war. Da elterliche Fürsorge kostspielig ist, wirft dies die Frage auf, warum Weibchen, die mit aggressiven Männchen verpaart sind, bereit sind eine höheren Anteil der Fürsorgelast zu tragen. Möglicherweise bieten aggressivere Männchen ihren Partnerinnen andere Vorteile, z.B. hochwertige Territorien oder gute Gene, oder investieren mehr in andere Aspekte elterlicher Fürsorge, wie z.B. Verteidigung der Jungen gegenüber Nesträubern.

Wir fanden tatsächlich Hinweise darauf, dass Blaumeisenpaare verschiedene Fürsorgeaufgaben untereinander aufteilen. Wurden Paare mit einem Nesträuber konfrontiert, investierte ein Partner verstärkt in Nestverteidigung, während der andere fortfuhr die Jungen zu füttern (Kapitel 2). Eine solche Arbeitsteilung könnte helfen das unmittelbare Überleben der Jungen zu sichern, während gleichzeitig langfristige negative Konsequenzen von Fütterunterbrechungen auf das Wachstum und die Verfassung der Jungen minimiert werden und somit die Fitness maximiert. Möglicherweise tragen aggressive Männchen einen höheren Anteil der Belastung der Brutverteidigung gegenüber Nesträubern, während ihre Partnerinnen mehr Aufwand in die Fütterung der Jungen stecken.

Kapitel 4 zeigt dass das Erkundungsverhalten von Kohlmeisen unter Selektion steht und das Selektionsdrücke zeitlich schwanken und sich zwischen den Geschlechtern unterscheiden. In Weibchen wurde schnelles Erkundungsverhalten in beiden Jahren selektiert, aber die Persönlichkeit mit Reproduktionserfolg verknüpfenden Pfade unterschieden sich zwischen den Jahren. In einem ‘guten’ Jahr, mit hoher Futterverfügbarkeit, produzierten schnell erkundende Weibchen größere Bruten, aber fütterten ihre Jungen später nicht häufiger als langsame Erkunder mit vergleichbaren (manipulierten) Brutgrößen. Schnell erkundende Weibchen produzierten insgesamt mehr, aber leichtere Jungvögel. Dies deutet an, das ungleiche Erkundungtypen den Trade-off zwischen der Anzahl der erfolgreich aufgezogenen Jungen und ihrer körperlichen Verfassung unterschiedlich lösten. Im Gegensatz dazu, produzierten schnell erkundende Weibchen in dem ‚schlechten‘ Jahr mehr Jungen in besserer Verfassung. Dies impliziert, dass schnell erkundende Weibchen relativ gut mit den schlechten Umweltbedingungen dieses Jahres zurecht kamen. Interessanterweise wurde diese Verknüpfung weder durch die ursprüngliche Brutgröße noch durch höhere Fütterraten vermittelt. Dies suggeriert dass andere, nicht in unserer Studie berücksichtigte Mechanismen an der Vermittlung von Verhaltens-Fitness Korrelationen beteiligt sind. Zum Beispiel könnten schnell

erkundende Weibchen ihren Reproduktionserfolg erhöhen, indem sie mehr in Nestverteidigung oder andere Formen von elterlicher Fürsorge, wie z.B. Wärmen der Nestlinge, investieren.

In männlichen Kohlmeisen stand Erkundungsverhalten nur in dem schlechten Jahr unter Selektion und Selektionsdrücke unterschieden sich zwischen den experimentellen Brutgrößenkategorien. Langsame Erkunder produzierten mehr und schwere Jungen wenn ihre ursprüngliche Brutgröße nicht verändert wurde. Mit reduzierten Brutgrößen hatten sie jedoch leichtere Jungen im Vergleich zu schnellen Erkundern. Diese Verbindung wurde nicht durch persönlichkeits-spezifisches Fütterverhalten vermittelt. Wir fanden jedoch Hinweise darauf, dass langsame Erkunder einen höheren Anteil an hochwertigen Beuteobjekten, d.h. Raupen, zum Nest bringen, was ihren höheren Fortpflanzungserfolg erklären könnte. Die Erkenntnis das die auf Erkundungsverhalten wirkenden Selektionsmuster geschlechts-spezifisch sind, ist im Einklang mit früheren Kohlmeisenstudien und verlangt nun erhöhte Aufmerksamkeit.

Zusammenfassend zeigt meine Arbeit, dass elterliche Fürsorge eine wichtige Rolle in der Verlinkung von Persönlichkeit mit Fitness spielt. Die gezeigten Pfade scheinen jedoch nicht allgemein gültig zu sein, sondern unterscheiden sich zwischen Arten, Jahren und Geschlechtern. Meine Doktorarbeit liefert somit einen wichtigen Beitrag für unser Verständnis wie natürliche Selektion Verhalten formen und Verhaltensvariation erhalten kann. Im Weiteren liefert meine Arbeit wichtige Hinweise darauf, dass andere, im Rahmen meiner Doktorarbeit nicht berücksichtigte Mechanismen, an der Vermittlung von Verhaltens-Fitness-Zusammenhängen beteiligt sind und hebt somit die Bedeutung von weiterführenden Studien in diesem Gebiet hervor.

General Introduction

Ever since Darwin, evolutionary biologists have been fascinated by understanding variation in phenotypic traits (e.g. Lack 1961; Endler 1986; Wilson 1998). This is not surprising given that variation provides the raw material on which selection can act on and is thus a prerequisite for adaptive evolution. Early studies investigating variation have largely focused on fixed traits, e.g. bill size or wing length, providing a variety of explanations for their evolutionary maintenance. Mechanisms and selective processes that can maintain phenotypic variation in fixed traits include mutation-selection balance, heterozygous advantage, fluctuating selection, frequency-dependency, life-history trade-offs and correlational selection (e.g. Stearns 1992; Hallgrímsson & Hall 2005). Yet, not all traits are fixed; some vary within and between individuals, so-called labile phenotypic traits (cf. Dingemanse & Dochtermann 2013). Examples for such labile traits are hormones and behaviours. While classic theories may account for the maintenance of between-individual variation in fixed traits, they cannot fully explain between-individual variation in labile traits, because these explanations do not elucidate why individuals are consistent versus plastic (Stamps 2007). This insight has led to a new field in behavioural ecology that aims to provide fully adaptive explanations for individual variation in behaviour (Dall et al. 2004).

Levels of variation in behaviour

Variation in behaviour can occur at multiple levels. Individual may differ in their average expression for single behaviours, in suites of functionally distinct behaviours or in behavioural plasticity (Dingemanse & Wolf 2010). First, individuals differ in

average behaviours. Similar to variation in fixed traits, this form of behavioural variation occurs at the between-individual level. Such consistent individual variation implies stability over time and/or across contexts (Dingemanse & Wolf 2010) leading to repeatable variation. Second, individuals vary their behaviour as a function of the environment, causing within-individual variation called individual plasticity (Nussey et al. 2007). The study of behaviour requires the integration of variation in average behaviour and behavioural plasticity within the same framework. This may be studying behaviour in terms of reaction norms, where the behavioural response of an individual over an environmental gradient, instead of the actual behaviour of an individual, is explicitly considered (Sih et al. 2004b; Smiseth et al. 2008; Dingemanse et al. 2010; Réale & Dingemanse 2010). This approach is called a 'behavioural reaction norm approach' (Smiseth et al. 2008; Dingemanse et al. 2010), where a *behavioural reaction norm* describes an individual's function relating its behavioural phenotype to the environment (Dingemanse et al. 2010). Classic examples for behavioural reaction norms include parental provisioning rate as a function of offspring begging intensity (Smiseth et al. 2008), and adjustments in anti-predator behaviour over different levels or predation risk (Sih et al. 2003; Quinn & Cresswell 2005).

Figure 1a illustrates the concept of 'behavioural reaction norms' for three different individuals (depicted by the blue, green and red lines). Although individuals change their behaviour across the environmental gradient (indicated by the non-zero slope), the rank order differences between individuals are maintained, thus indicating that there are *consistent between-individual differences* in behaviour (Martin & Réale 2008; Dingemanse et al. 2010; Stamps & Groothuis 2010). These consistent differences between individuals are reflected in the elevations (intercepts) of the reaction norms (Figure 1a) and are commonly referred to as '*animal personalities*' in the behavioural ecology literature (Dingemanse et al. 2010; Réale et al. 2010).

Within this framework, individual differences in response to the environment are

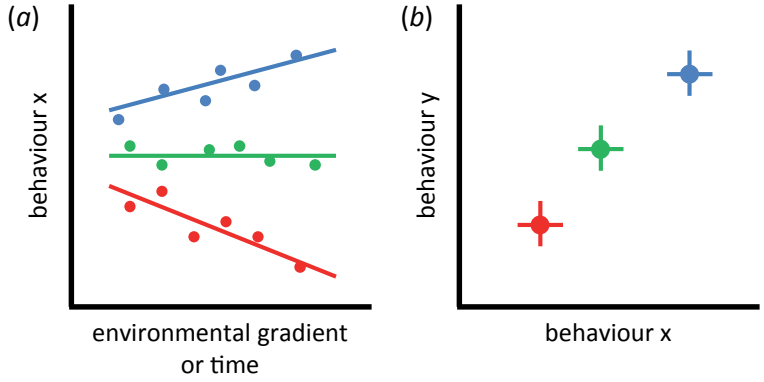

Figure 1. Levels of variation in behaviour. Different individuals are represented by different colours (blue, green, red). Panel (a) visualizes *consistent individual variation in a single behaviour.* Dots represent repeated behavioural measures for each individual along an environmental gradient. Lines represent behavioural reaction norms. The presence of individual differences in behaviour is illustrated by the different elevations (i.e. the statistical 'intercepts') of the reaction norms. The non-overlapping reaction norms imply that rank order differences in behaviour between individuals are maintained over the environmental gradient (i.e. contexts), reflecting behavioural consistency. *Individual differences in behavioural plasticity* are visualized by the variation in the slope of the different reaction norms. Panel (b) illustrates *consistent individual variation in suites of correlated behaviours* (i.e. 'behavioural syndromes' sensu Dingemanse et al. 2012a). Dots depict correlations between the means of behaviour x and y over various (>1) observations. Vertical and horizontal lines indicate that both behaviours vary within individuals, because of behavioural plasticity and/or measurement error (cf. Dingemanse & Dochtermann 2013). The figure is modified from Figure 1 in Dingemanse & Wolf (2010).

also explicitly considered. This *within-individual variation* in behaviour, referred to as *behavioural plasticity* (Nussey et al. 2007; Dingemanse et al. 2010), is reflected in the different slopes of the reaction norms (Figure 1a). In the depicted scenario (Figure 1a), individuals differ both in their responsiveness *and* in their average levels of behaviour resulting in a covariation between these two levels of variation, called 'personality-related plasticity' (Mathot et al. 2012). In mice, for example, some individuals adjust their aggression level as a function of social context, whereas others do not (Koolhaas et al. 1999).

Levels of covariation in behaviour

Individual differences are commonly correlated between behavioural traits, resulting in between-individual variation in suites of correlated behavioural traits (Figure 1b; Bell 2007; Réale et al. 2007). Here, non-zero behavioural correlations exist between (as opposed to within) individuals, referred to as a '*behavioural syndrome*' (Dingemanse et al. 2012a). For example, individuals that are on average (i.e. over multiple observations) more aggressive often also explore a novel environment on average more rapidly compared to individuals that are on average less aggressive. Such an aggression-exploration syndrome has been demonstrated for a wide array of taxa, including bird, fish, mammals and insects (Garamszegi et al. 2012). Yet, a correlation between two phenotypic traits in the population cannot readily be interpreted as evidence for a syndrome. This is because such phenotypic correlations are composed of two different aspects, namely a between- and a within-individual component, which are underpinned by different biological mechanisms, respectively (cf. Dingemanse et al. 2012a; Brommer 2013; Dingemanse & Dochtermann 2013).

Between-individual correlations result from differences in average behaviours between individuals (cf. Figure 1a), caused by variation in both the genetic constitution and permanent environment effects and may thus reflect underlying genetic correlations (Dochtermann 2011). Between-individual correlations among behavioural traits may thus impose constraints to the independent evolution of behaviours (Lande & Arnold 1983; Price & Langen 1992; Bell 2005; Dochtermann & Dingemanse in press), regardless of whether they had evolved by natural selection (cf. linkage disequilibrium). Indeed, a recent meta-analysis has shown that syndrome structure is in fact constraining behavioural evolution by 33% (Dochtermann & Dingemanse in press). Behavioural ecologists are thus seeking to understand why such between-individual correlations might have evolved in the first place using the models that I detail below.

Within-individual correlations, in contrast, arise through correlated plastic behavioural responses to environmental conditions (Dingemanse & Dochtermann 2013), thus mainly reflecting the integration of plasticity among multiple behaviours. For instance, this would be the case when an individual changes its aggressiveness and activity in concert from one context (e.g. foraging) to the next (e.g. territory defence). As repeatability of behaviour is relatively moderate (0.37; cf. meta-analysis by Bell et al. 2009), most of the correlation at the phenotypic level is due to the so-called within-individual correlation (Dingemanse et al. 2012b). Hence, studies interested in syndromes (i.e. between-individual correlations) must design studies in such a way that the within-individual correlation is partitioned out. Throughout my thesis, whenever talking about 'personality', I explicitly refer to between-individual differences in reaction norm intercepts.

Why are there consistent individual differences in behaviour?

Even though it is now commonly acknowledged that animals from a wide array of taxa show consistent between- and within-individual differences in behaviour, it is far less clear why these differences actually exist (Dall et al. 2004; Sih et al. 2004a; Sih et al. 2004b; Dingemanse & Réale 2005; Stamps 2007; Sih & Bell 2008; Wolf & Weissing 2010). To acquire insight into the maintenance, functional significance and evolution of such consistent individual differences in behaviour, we need to answer the questions of why individuals from the same population differ in their average level of behaviour and why individuals behave in a consistent way over time, i.e. why behaviour is repeatable instead of all individuals showing unlimited phenotypic plasticity (Dall et al. 2004; Sih et al. 2004b; Dingemanse & Réale 2005; Stamps 2007).

Classic explanations for the maintenance of behavioural variation

The most frequently suggested selective mechanisms for the conservation of behavioural variation include heterogeneous forms of selection, such as temporal or spatial fluctuation in selection pressures, sexually antagonistic selection and frequency dependent selection, and other types of selection such as correlational selection and life-history trade-offs. These explanations for the maintenance of variation in behaviour are based on the assumption that the behaviour of an individual – similar to morphological traits – becomes (at some point in its development) fixed and can thus only account for the co-existence of different behavioural phenotypes (Stamps 2007). Yet, they do not explain why individuals of the same species consistently differ in their behaviour over time and across context and do not show unlimited behavioural plasticity (Réale et al. 2007). We therefore call those mechanisms, detailed below, 'classic' explanations.

(i) *Spatio-temporal fluctuating selection*. This type of heterogeneous selection can occur when environmental conditions (e.g. availability of food or other limited resources) differ in space and/or time thus creating heterogeneous selection pressures on a trait. Under this scenario, the total effect of selection on a trait might even out resulting in no net selection. Empirical studies investigating selection on behavioural traits indicate that heterogeneous selection commonly occurs in a wide array of taxa (this form of selection was reported in 10 out of 11 studies, i.e. 91%; cf. Dingemanse & Réale 2013). For example, spatial variation in selection has been demonstrated in a study on great tits, *Parus major*, where individuals with fast exploratory tendency had a higher reproductive success in low-density habitats compared to slow-exploring individuals (Quinn et al. 2009).

(ii) *Sexually antagonistic selection*. This form of fluctuating selection can occur when males and females differ in their optimal values for the same trait with this trait at the same time being positively genetically correlated across sexes because the genes coding for the trait are (in part) independent of sex (Cox & Calsbeek

2009). As males and females are likely to experience different (social) environments and have to face different challenges (e.g. energetic and nutritional requirements), their behaviour might also have different fitness consequences. For example, in great tits, slow-exploring males tended to survive better in certain years, whereas at the same time, selection favoured fast exploratory tendency in females and vice versa (Dingemanse et al. 2004). Another striking example stems from a study on a sequentially hermaphroditic fish species that demonstrated the existence of tight cross-sex correlations for two behavioural traits with assumed sex-specific optima (Sprenger et al. 2012).

(iii) *Negative frequency-dependent selection*. Heterogeneous selection can also act in a frequency-dependent manner, when the fitness outcome of a certain phenotype depends on its abundance relative to other phenotypes in the same population (Maynard Smith 1982; Roff 1998). In the case of negative frequency dependent selection, the proportion of certain behavioural phenotypes should be negatively related to selection acting on this specific behavioural type. Some evidence for a particular form of negative frequency-dependent selection comes from studies on great tits, where the social environment affected reproductive success. While assortatively paired males lost most paternity in their own nest (van Oers et al. 2008), assortatively paired individuals were also shown to have offspring in best condition (Both et al. 2005) and to have the highest number of recruits (Dingemanse et al. 2004).

(iv) *Correlational selection*. This form of selection takes place when individuals with particular combinations of traits have the highest fitness, i.e. if selection acts on the link between different behavioural traits. This can result in a fitness 'ridge' where several multivariate phenotype combinations do equally well (Brodie 1992; Brodie et al. 1995; Sinervo & Svensson 2002), thereby maintaining variation in these behaviours. So far, only a few studies have attempted to estimate correlational selection acting on the link between different behaviours (Réale & Festa-Bianchet

2003; Bell & Sih 2007; Boon et al. 2007; Réale et al. 2009; Adriaenssens & Johnsson 2013). These studies generally failed to provide evidence for correlational selection playing a role in maintaining behavioural syndromes, which may in part reflect lack of statistical power (Kingsolver et al. 2001). Yet, correlational selection has been shown to favour the correlation between two behavioural traits (neuroticism and extraversion) in humans (Eaves et al. 1990).

(v) *Life-history trade-offs*. Mechanisms involving life-history trade-offs have long been implied to maintain genetic variation (Roff 2005) and have also been suggested to maintain variation in behaviour (Wolf et al. 2007; Biro & Stamps 2008; Réale et al. 2009). This is because behaviours that are likely to affect the probability of acquiring resources while simultaneously increasing the mortality risk are expected to coevolve with general life-history strategies (Dingemanse & Réale 2013). Trade-offs that have been suggested in the context of behaviour, include the trade-off between growth and mortality (Stamps 2007), early and late reproduction (Wolf et al. 2007) and alternative reproductive strategies (such as within-pair versus extra-pair paternity (Patrick et al. 2012). Aggressive and explorative individuals, for example, are expected to acquire high-quality resources and are therefore able to reproduce early in life. At the same time they face a higher mortality risk compared to less aggressive individuals and are therefore expected to have a relative short reproductive period. Support for this hypothesis comes, for example, from a study on bighorn sheep, *Ovis canadensis*, where aggressive individuals reproduced relatively early, but at the same time had a short life-span, whereas the opposite was true for less aggressive individuals (Réale et al. 2009).

Adaptive explanation for the maintenance of repeatable variation

The previous section provides explanations for the existence of behavioural variation in general. Yet, there is growing appreciation for the notion that the existence of personality differences requires addressing this question specifically at the

between-individual level. Thus, in this section, I will briefly consider more integrative and complete explanations for the maintenance of repeatable behavioural variation. Models for adaptive personality differences can be divided into three non-exclusive categories (Wolf & Weissing 2010). The first category includes adaptive models explaining how differences in state can result in consistent between-individual differences in behaviour. State in this context refers to all kind of characteristics of an individual: its size or morphology, its physiological and physical conditions (e.g. amount of energy reserves), but also to features of its current environment, e.g. type of habitat or social environment (Dall et al. 2004; Wolf & Weissing 2010). Moreover, state can also be a genetic attribute of an individual. The second group comprises models investigating how positive feedback loops between state and behaviour can strengthen existing initial individual differences over time. Finally, models not involving differences in state form a third category. Here, I detail the basic reasoning behind the state-dependent theoretical models for adaptive between-individual variation in behaviour ('adaptive personalities').

The underlying assumption of state-dependent models is that individuals differ in state. Such state differences in turn can impact and limit the behaviours that an individual is able to perform as well as its costs and benefits, resulting in state-dependent behaviours (Wolf & Weissing 2010). Even for individuals that are genetically identical, such state-dependent behaviour can result in consistent between-individual differences (Dall et al. 2004; Wolf & Weissing 2010). For instance, an individual with low energy resources might not be able or willing to invest in reproduction, but instead might have high benefits from engaging in foraging behaviour even under high risk of predation. Yet, while state variables that are inherently stable, e.g. organ size or basal metabolic rate, can easily explain the existence of *consistent* differences in behaviour, understanding how other, more labile, state variables can result in repeatable behavioural variation requires an additional explanation (i.e. must be part of the theoretical framework). This is because a behaviour that is dependent on a specific state will only be consistent over

time if the underlying state itself is relatively stable too (Wolf & Weissing 2010). For instance, labile states could remain relatively stable, if the current behaviour of an individual impacts its future state and thereby its subsequent behaviour. Positive feedback mechanisms would lead to long-term consistency and further divergence of initially small differences in labile states and their associated state-dependent behaviours (Rands et al. 2003; Dall et al. 2004; Sih & Bell 2008; Luttbeg & Sih 2010; Wolf & Weissing 2010). In contrast, state-dependent models that are based on negative feedback mechanisms can explain consistency in behaviour only over relatively short periods of time (Luttbeg & Sih 2010).

Importantly, state-dependency of behaviour is also the underlying basis for a variety of other theoretical models explaining repeatable variation in behaviour, including life-history trade-off models (Stamps 2007; Wolf et al. 2007; Biro & Stamps 2008). Trade-offs between productivity and mortality, for example, can explain repeatable variation in behaviour only, if individuals also consistently differ in growth and fecundity over their entire life span. Indeed, empirical evidence suggests that animals typically show consistent variation in growth and fecundity rates (e.g. Zera & Harshman 2001; Nussey et al. 2006; Stamps 2007), which are based on consistent differences in the physiological and morphological machinery (e.g. the size of organs related to food and energy metabolism, basal metabolic rate or the endocrine system) that controls an individual's growth or fecundity (Biro & Stamps 2008). Moreover, trade-offs between current and future reproduction that can generate consistent individual differences in behaviour (Wolf et al. 2007) are also likely to be underlined by differences in states.

Proximate mechanisms underpinning behavioural variation

The previous paragraph clarifies that individual differences in state, including physiological mechanisms, are likely to play an important role in underpinning consistent behavioural variation. Thus, in order to fully understand why animals

consistently differ in their behaviour from an adaptive perspective, it is also important to identify the underlying proximate mechanisms that mediate this variation. The expression of a particular behavioural phenotype is regulated by a control system, the neuroendocrine system, which might differ between individuals, thereby constraining the behavioural range that each individual is able to express. Moreover, it has been argued that a functional approach alone is not sufficient to explain the behaviour of individuals (McNamara & Houston 2009). Thus, only when integrating proximate and ultimate explanations, we might better increase our understanding of the existence of behavioural variation.

There is extensive evidence that individual variation in behaviour is linked proximately to differences in the genetic and neuroendocrine system among individuals (Koolhaas et al. 1999; Groothuis & Carere 2005; Penke et al. 2007; van Oers & Mueller 2010). As hormones typically act on several target tissues simultaneously, they represent likely candidates for mediating the expression of single behaviours but also of suites of correlated behavioural traits (Ketterson & Nolan 1999) and could thus also explain the occurrence of behavioural syndromes (Sih et al. 2004b; Bell 2007). Hormones can control behavioural modifications by modulating sensory inputs, by acting directly on the central nervous system or by regulating effector systems (Ball & Balthazart 2008). Although there is evidence for all of the three mentioned mechanisms, the most important targets of hormone action are located in the brain. After entering target cells in the brain, hormones can affect neural processing (by binding to intracellular receptors and enhancing the transcription of particular genes), thereby affecting the expression of certain behaviours. For example, the steroid hormone testosterone has repeatedly been shown to affect between- as well as within-individual variation in a variety of behaviours in male birds, such as aggressiveness, reproductive behaviour and parental care (Ketterson & Nolan 1999). Yet, relatively few studies have explicitly investigated the role of androgens in mediating repeatable differences in behaviour, i.e. whether consistent between-individual variation in testosterone levels might

be responsible for causing consistent behaviour differences (e.g. King 2002; Sellers et al. 2007; Mutzel et al. 2011). Moreover, to our knowledge, there is no study that investigated the link between hormones and behaviour simultaneously at the within- and between-individual level by combining an observational and experimental approach. We therefore address this issue in chapter 1. Within-individual correlations between hormones and behaviour come about, when changes in hormone concentrations (e.g. increase of testosterone in the breeding season or after testosterone implantation) cause subsequent changes in behaviour of the same individual (e.g. increase of aggressiveness). Yet, to test whether hormones underpin repeatable between-individual variation in behaviour, it is necessary to investigate whether individuals with on average lower hormone concentrations also show on average lower levels of behaviour. However, the behaviour of an individual might also affect its hormone level (Koolhaas et al. 2010), making it difficult to draw definite conclusions about the causality of detected links between hormones and behaviour.

One difficulty with studying testosterone-behaviour relationships is that there are multiple pathways via which hormones can mediate between-individual variation in behaviour. First of all, individuals might differ in circulating plasma testosterone levels and therefore express different levels of behaviours. Such variation in plasma testosterone concentrations can be responsible for causing within- as well as between-individual differences in behaviour. Whereas quantifying differences in testosterone blood titers is relatively straight forward, there are other, more 'hidden', pathways that cannot be assessed that easily. For instance, there might be genetically determined between-individual variation in testosterone receptor density, specificity and affinity, affecting how strongly a given testosterone level can acts on a certain target tissue (Adkins-Regan 2005; Ball & Balthazart 2008). Moreover, heritable variation in plasma-binding proteins can substantially affect the availability of a certain hormone (Dufty et al. 2002; Ball & Balthazart 2008). The regulation of any of these steps could differ between individuals, thus causing

between-individual variation in the response to equal amounts of circulating testosterone concentrations (Ball & Balthazart 2008). Yet, in contrast to circulating testosterone levels that can show high fluctuations within- and between individuals, the flexibility of these alternative mechanisms is likely to be limited within individuals, thus probably playing an important role mainly for causing between-individual differences in behaviour. Thus, when only measuring circulating plasma hormone levels, such alternative pathways might obscure potential hormone-behaviour links at the between-individual level. One possible option to diminish confounding effects of such alternative pathways is to experimentally manipulate hormone titers, e.g. through hormonal implants, and then comparing the behaviour of individuals before during and after the manipulation (within-individual level) and/or with the behaviour of control groups (between-individual level) (Ketterson & Nolan 1999). In chapter 1 of this thesis, we address the question of whether testosterone underpins consistent within- and between individual variation in exploratory behaviour by using observational and experimental approach.

Mechanistic pathways linking behaviour with fitness

Adaptive explanations for the existence of consistent variation in behaviour imply that natural selection favours the evolution and maintenance of this variation. Thus, to understand how natural selection acts on behaviour it is necessary to identify potential mechanistic pathways linking behaviour with fitness. Whereas direct links can be quantified with a phenotypic selection approach, measuring indirect pathways requires the use of path analysis (Figure 2). In the chapters 3 and 4 of my thesis, I thus investigate potential mechanistic pathways linking behaviour with fitness by applying such path analyses. In chapter 4, I additionally apply a phenotypic selection approach to estimate the direct selection pressures acting on behaviour.

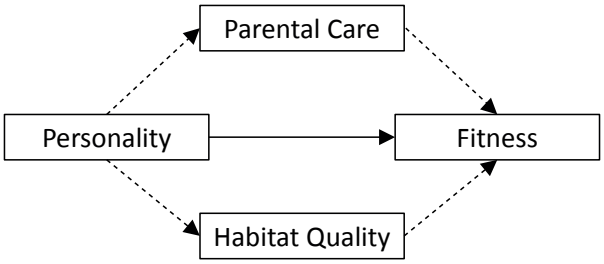

Figure 2. Direct and indirect links between personality and fitness. The solid arrow illustrates a direct link, indicating the presence of mechanistic pathways not considered in the model. The dashed arrows illustrate the presence of indirect links between personality and fitness, mediated via other variables, here 'parental care' and 'habitat quality', respectively. Path analysis allows the calculation of the strength of each direct and indirect pathway while simultaneously controlling for effects of the other variables in the model. The phenotypic selection approach estimates the strength of the overall selection pressures acting on personality, i.e. the sum of all pathways.

Direct links between behaviour and fitness

There are various ways to estimate natural selection, but one of the most commonly used methods is the phenotypic selection approach (Lande & Arnold 1983). This approach measures the covariance between traits and relative fitness estimates, revealing both the direct force (Lande & Arnold 1983) and the shape (Brodie et al. 1995) of selection on a given trait, while at the same time controlling for indirect selection effects of other correlated traits (Lande & Arnold 1983). Another advantage of this estimation method is that it allows the direct comparison of the strength of selection gradients between different traits, datasets, years, and/or sexes by standardizing traits within years and sexes prior to analysis (Arnold & Wade 1984). As behavioural traits are often correlated with each other and selection pressure are likely to fluctuate, this makes selection analysis a particularly suitable tool for estimating selection on behavioural traits (Réale et al. 2000). Yet, there are also limitations to phenotypic selection analysis. For example it does not allow controlling for environmental variation among individuals, which can strongly bias measures of phenotypic selection (Scheiner et al. 2002), nor does it reveal

mechanistic pathways via which behaviour might affect fitness, hampering our understanding of *how* exactly selection acts on behaviour. Thus, only by considering indirect links between personality and fitness might we further our understanding of the underlying mechanisms linking behaviour with fitness. The quantification of potential indirect pathways is therefore a central theme in this thesis.

Indirect links between behaviour and fitness

Path analysis represents a particular form of multiple regression that allows estimating the direction and the strength of directional relationships for complex models with multiple interdependent variables (Scheiner et al. 2000; Grace 2006). The causal relationships of a path model are typically hypothesized *a priori* by considering theoretical predictions or assumptions or by using the knowledge of previous studies (Scheiner et al. 2002; Grace 2006). The big advantage of path analysis is that it allows calculating partial correlations coefficients (path coefficients) between two variables while simultaneously controlling for effects of all other variables included the model (Grace 2006). This makes path analysis a powerful tool to separate direct from indirect effects (i.e. produced by effects of other correlated variables).

Mechanistic pathways linking personality with fitness are likely to involve parental care behaviours, such as offspring provisioning and nest defense behaviour. This is because in many organisms, caring for young is essential for the survival of the offspring. Thus, the amount of parental care that an individual is willing or able to provide to its offspring will most likely influence its reproductive success. At the same time parental care also incurs costs for the parents, resulting in a trade-off between the benefits (e.g. increased survival of the current brood) and costs (e.g. decreased future breeding potential) of high levels of parental care (Trivers 1972). In systems with biparental care (in most bird species), optimal investment strategies become even more complex, as parents have to negotiate about the amount of care that each partner is willing to provide (Johnstone & Hinde 2006). In the chapters 3

and 4 of my thesis, I therefore address the question of whether variation in fitness between different personality types is caused by these types differing in patterns of parental care.

Offspring provisioning

One of the most intensively studied aspects of parental care is offspring provisioning, which typically serves for investigating adaptive parental investment (Clutton-Brock 1991; Royle et al. 2012), biparental cooperation (Houston et al. 2005; Harrison et al. 2009), parent-offspring conflict and the evolution of begging signals (Wright & Leonard 2002; Hinde & Kilner 2007; Smiseth et al. 2008). The most commonly used proxy for parental effort is provisioning rate, which implies that feeding rate is a good reflector of the amount of food delivered to nestlings. Yet, studies investigating parental provisioning behaviour in more detail found that feeding rate does not always adequately reflect the amount of biomass and thus parental effort. Instead, parents do not only differ in how often they visit the nest, but can also show within- and between-individual variation in the size of the prey delivered to nestlings (Wright et al. 1998; Naef-Daenzer & Keller 1999; Grieco 2001; Mägi et al. 2009). Thus, even though in some cases feeding rate on its own might be a good measure of parental effort, only in combination with load size it can give a definite and reliable estimate of the amount of biomass brought to the nest. In addition, comprehensive studies on provisioning behaviour should also consider prey type, as there is evidence that prey items can greatly differ in their nutritional and energetic value and that parental foraging strategies can also involve changes in the type or range of prey items fed to the young (Royama 1966; Tinbergen 1981; Wright & Cuthill 1989, 1990; Wright et al. 1998; Grieco 2001). While I use feeding rate as a proxy for parental provisioning behaviour in chapter 3 of my thesis, chapter 4 also includes measures of prey size and prey type.

Figure 3. Great tit provisioning nestlings with a crane fly of the family Tipulidae, a common prey type that Bavarian great tits deliver to their nestlings.

Nest defence

Besides nestling provisioning, defence of offspring against predators is another important from of avian parental care (Shields 1984). As predation of offspring is an important cause of nest failure in birds (Ricklefs 1969), nest defence can greatly increase nestling survival (Greig-Smith 1980; Blancher & Robertson 1982; Montgomerie & Weatherhead 1988; Markman et al. 1996). Yet, it also incurs costs for parents and offspring (Lima 2009). Whereas parents risk being depredated or injured, offspring might also pay a cost in terms of increased risk of starvation or reduced growth, because parents have to interrupt feeding during nest defence behaviour. In order to maximize the reproductive output of the current nesting attempt, while taking into account effects on their future productivity (i.e. their ability to invest in future broods (Trivers 1972)), parents therefore have to trade-off nestling provisioning and nest defence in an optimal manner. For example, parents could try to adjust provisioning activity over time according to variation in the current nest predation risk levels. Another way to solve this dilemma, would be to divide the different tasks of parental care within pairs, i.e. one parent continues to

Figure 4. A female great tit defending her brood against a nest predator (the great spotted woodpecker).

feed the nestlings, while the other defends the nest. There might also be consistent between-individual variation in how much time and energy is invested in nest defence and/or feeding of nestlings, which in turn is likely to be linked to other behavioural traits outside the parental care context. The temporal trade-off between offspring provisioning and nest defense behaviour is addressed in chapter 2 of my thesis.

Study species and study areas

For my dissertation, I used three different study species. The house sparrow, *Passer domesticus*, is a small year-round resident belonging to the family Passeridae. It can be found in most parts of the world and in all kind of habitats, thus making it the probably most widely distributed species (Anderson 2006). Its occurrence is strongly associated with human habitations in urban and rural areas and they readily accept artificial nestboxes for roosting and breeding. One particular characteristic of house sparrows is that the bill colouration of male birds varies from pale horn to jet-black. Whereas the bills of males are usually pale coloured outside the breeding season, this colour gradually darkens towards the breeding season (Witschi &

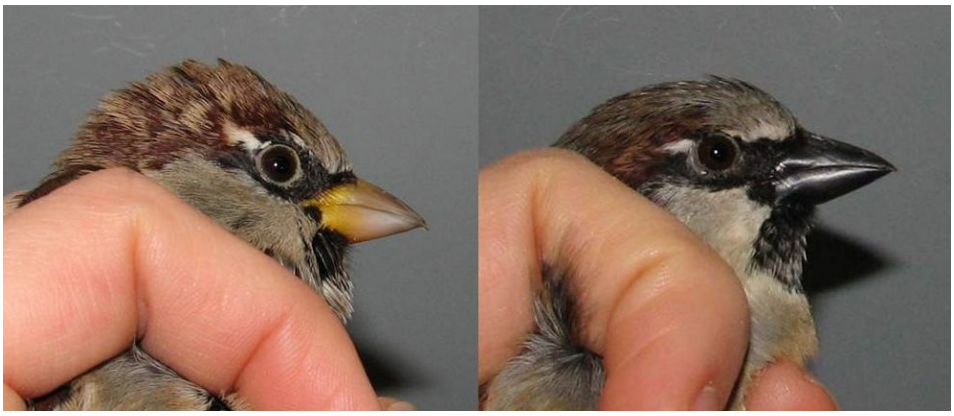

Figure 5. Bill colouration of male house sparrows. Natural occurring bill colouration ranges from a pale horn (left side) to jet black (right side).

Woods 1936). Moreover, this variation is linked to prevailing circulating plasma testosterone levels, making bill colouration a reliable short-term predictor of T levels (Keck 1933; Witschi 1936; Pfeiffer et al. 1944). The possibility to use two different indices for plasma testosterone levels (direct point estimates from blood samples and 'running average' levels of testosterone levels from the bill colour) makes this species particularly suitable for studying testosterone-behaviour relationships. Moreover, the experimental design of the study required repeated behavioural and hormone measures, which would have been a challenging task in a wild population.

The blue tit, *Cyanistes caeruleus*, and the great tit are two closely related non-migratory passerines from the family Paridae that are widely distributed across Europe and Asia. While great tits can be found in all kind of habitats, including deciduous, coniferous and mixed woodland, forest edges and urban areas, blue tits mainly occur in deciduous or mixed woodland with a high proportion of oak. Both species are seasonally monogamous with bi-parental care and readily accept nest boxes for breeding. This makes them the ideal species for monitoring reproductive success and repeatedly quantifying behavioural traits. The clutch size of blue tits mostly ranges between 7-13 eggs whereas great tits typically lay between 3-12 eggs.

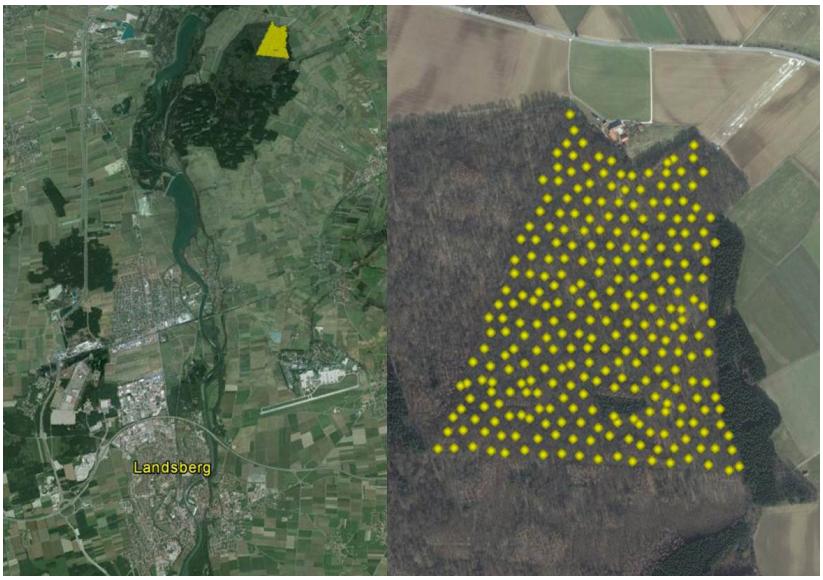

Figure 6. Blue tit study area. Left side: Location of the study area in 'Westerholz' near Landsberg am Lech in southern Germany. Right side: Close up view of the 40 ha area containing 277 nestboxes, placed approximately 40 m apart. The entrance hole of the nestboxes has a diameter of 26 mm, thereby precluding the common great tit and other hole-nesting species from breeding in the nestboxes. The study area is located in an unmanaged part of the forest and mainly consists of mature oak trees, *Quercus* sp.. (Source: Google Earth)

Figure 7. Great tit study area. The map shows the 12 plots, with each study site consisting of 50 nestboxes (yellow dots) arranged on a 50 x 50 m grid. Taken together the plots cover a total area of about 120 ha of mixed woods with beech, *Fagus sylvatica*, as the predominant tree species. (Source: Google Earth)

Figure 8. Typical habitats of the blue tit and great tit study area. Above: Unmanaged major oak tree forest of the blue tits study area. Below: Mixed forest with beech tree as the predominant species and relatively dense understory, typical for major parts of the great tit study area.

Aims of the thesis

Even though theoreticians have provided various explanations for why animal personalities and behavioural syndromes might result from natural selection (Dall et al. 2004; Dingemanse & Wolf 2010; Wolf & Weissing 2010), only few studies have yet explicitly tested their predictions and assumptions. As a consequence, the reasons for why personalities and syndromes persist in nature are still largely obscure. This thesis aimed to shed more light on the process of behavioural evolution by providing deeper insights into physiological mechanisms underpinning variation in behaviour on the one hand (chapter 1) and into pathways by which behavioural variation is translated into fitness on the other hand. In chapter 2, we address how parents resolve the trade-off between provisioning behaviour and nest defence to maximize fitness. In chapter 3, we investigate the roles of parental feeding rates and lay date on mediating the link between male aggressiveness and male and female exploratory behaviour on reproductive success. In chapter 4, we investigate the role of parental provisioning behaviour in more detail by quantifying not only feeding rate but also other aspects of provisioning behaviour, i.e. prey size and prey type, and further explore whether mechanistic pathways linking exploratory behaviour and reproductive success are year-specific.

In **chapter 1**, we investigated proximate mechanisms underpinning within- and between-individual variation in exploratory behaviour with a joint observational and experimental approach. More specifically, we tried to answer the question of whether the androgen hormone testosterone could be involved in mediating between-individual differences in exploratory behaviour. First, we used observational data to test whether between-individual variation in exploratory behaviour was correlated with variation in two indexes of male testosterone level (see below). As a second step, we experimentally manipulated circulating plasma testosterone levels by applying testosterone implants to a subset of individuals and evaluated whether changes in behaviour within individuals were underpinned by changes in testosterone levels

and whether experimentally induced individual variation in T levels affected the amount of consistent individual difference in exploratory behaviour. This study was carried out on a captive population of male house sparrows. This species was particularly suitable for addressing these questions, as it allowed using two different indexes of male testosterone levels, therefore rendering a more complete picture of the link between testosterone and behaviour. While blood samples provided a direct point estimate of circulating plasma testosterone titre, the degree of melanization of the bill (i.e. bill colour) has been shown to be a reliable estimate for a recent 'running average' of circulating testosterone levels (Keck 1933; Witschi 1936; Pfeiffer et al. 1944; Laucht et al. 2010).

In **chapter 2** we explored temporal trade-offs between two important aspects of parental care, namely nestling provisioning and nest defence against predators across different levels of predation risk. We did this by quantifying the responses of breeding pairs towards a taxidermic model of a great spotted woodpecker, *Dendrocopos major*, a typical nest predator of cavity-nesting birds (Löhrl 1972), and then comparing parental provisioning behaviour before, during and after the encounter with the nest predator. Additionally, we examined whether parents were able to truly recognize a nest predator or whether they merely showed a general fear response towards a novel object close to their nest box, by comparing parental responses towards a woodpecker model with those shown towards a novel but non-threatening object (a red rubber ball). This study was carried out in a natural nest box population of blue tits, where typically both members of a pair participate in offspring provisioning and nest defense.

In **chapter 3** we aimed to reveal mechanistic pathways by which variation in personality might be translated into variation in reproductive success in a natural population of blue tits by applying a multivariate approach towards understanding variation. We quantified several variables likely to be involved in forming such pathways, namely parental provisioning rates, female lay date and brood size. First,

we measured two behavioural traits, exploration of a novel environment and male aggression towards a conspecific territorial intruder, which are typically considered to reflect personality in birds and that have previously been shown to be highly correlated in a variety of taxa (Garamszegi et al. 2013). Second, we quantified parental feeding rates as a proxy for provisioning behaviour. Third, we recorded lay date, brood size, number of fledglings and average fledgling mass of a brood as direct and indirect estimates for reproductive success. Based on previous literature, we then considered various direct and indirect pathways and tested the relative support of each of the *a priori* hypothesized links by applying path analysis.

In **chapter 4** we used a natural population of great tits to assess the link between exploratory behaviour and reproductive success and to measure selection acting on exploratory and provisioning behaviour. Yet, phenotypic selection can be biased by non-random environmental variation between phenotypes, i.e. when phenotypes consistently differ in the environment they choose to live in (Scheiner et al. 2002). For instance fast-exploring individuals might lay larger clutches than slow explorers because they breed in lower vs. higher quality habitats (cf. Both et al. 2005) and therefore consistently experience a different environment. Because our interest was in whether personality types differed in parenting behaviour *per se,* we therefore experimentally determined the brood sizes that these birds were given to raise. This experimental manipulation of brood sizes thereby forced some individuals to work either harder or less hard, enabling us to assess how parenting ability varied between personality types under easy vs. tough conditions. Moreover, by increasing the total variation and broadening the distribution of brood size, we could estimate the effects of fitness over a broader range of values, which facilitated the detection of actual links between behaviour and fitness (Schluter 1988). We first applied path analysis to identify specific pathways underlying personality-fitness relationships. In contrast to the study on blue tits (chapter 3), we did not focus on parental feeding rates as proxies of prey delivery, but instead directly quantified the size of each prey item and its type (e.g. caterpillar, other insect, spider, beech nut). This approach

enabled us to assess directly whether parental personality types differed in prey delivery *per se*.

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Chapter 1

Circulating testosterone levels do not affect exploration in house sparrows: observational and experimental tests

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Despite rapidly accumulating evidence for the existence of consistent individual differences in suites of correlated behaviours (i.e. 'animal personalities'), little is known about proximate mechanisms causing such variation. Individual variation in circulating levels of testosterone (T) is often hypothesized to underpin personality traits such as aggressiveness and exploratory behaviour. Here we provide a comprehensive test of this hypothesis. We quantified variation in exploratory behaviour of a novel environment in a captive population of wild-caught male house sparrows, *Passer domesticus*. We then investigated the relationship between the observed behaviours and circulating levels of T, using two approaches. First, we tested whether measures of exploratory behaviour correlated with (1) point-sampled plasma T levels and (2) T-dependent ornamentation (bill coloration) in 114 males. Neither direct nor indirect estimates of individual variation in T levels were correlated with the assayed behaviours. Second, we experimentally increased plasma T levels of 21 males with T implants, using 21 placebo-implanted males as a control group. Experimentally induced between-individual variation in T levels did not increase the amount of between-individual variation in exploratory behaviour. Our results thus strongly suggest that, in house sparrows, between-individual variation in circulating levels of T cannot serve as a causal explanation for the existence of individual variation in exploratory behaviour.

Animals constantly have to respond to changes in physical and social components of the environment. Whereas behavioural plasticity has long been assumed to represent an adaptive response to such environmental changes (Piersma & Drent 2003; Dall et al. 2004), accumulating evidence suggests that individuals do not show the full range of behavioural trait values present in their population (Réale & Dingemanse 2010). In other words, individuals often differ consistently in their behaviour over a range of environmental contexts (Dall et al. 2004; Sih et al. 2004; Réale et al. 2007). Such consistent individual differences in behaviour are referred to as 'animal personalities' (Dall et al. 2004; Réale et al. 2007; Dingemanse et al. 2010) and personality traits that are correlated are commonly referred to as 'behavioural syndromes' (Sih et al. 2004; Bell 2007; Réale et al. 2007). Despite abundant research focused on animal personalities, surprisingly little is known about the potential role of androgens as proximate mechanisms that underpin consistent individual variation in behaviour.

Consistent individual variation in behaviour might be linked proximately to individual differences in circulating hormone levels (Sih et al. 2004; Bell 2007; Réale et al. 2007). Baseline testosterone (T) levels in particular have been suggested to be a hormonal marker for individual differences in personality (Sellers et al. 2007). For instance, it has been shown that T influences the personality traits aggressiveness, boldness and activity (e.g. Wingfield et al. 1987; Koolhaas et al. 1999; Lynn et al. 2000), which jointly constitute a behavioural syndrome in birds (e.g. Verbeek et al. 1996; Drent & Marchetti 1999; Van Oers et al. 2004) and various other taxa (Réale et al. 2007). In addition, earlier observational and experimental studies indicated that T might be linked to behavioural persistency (Andrew 1972; Rogers 1974; Young & Rogers 1978). This trait is likely to be associated with the slow/fast personality type, as slow-exploring birds seem to show greater behavioural flexibility, whereas fast-exploring birds tend to stick to routine-like behaviour (Marchetti & Drent 2000). Finally, seasonal fluctuations in T levels

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(Hegner & Wingfield 1986) could potentially explain seasonal differences in exploration within individuals because both T levels and the tendency to explore seem to peak in spring (Dingemanse et al. 2002; Mettke-Hofmann 2007; Quinn et al. 2009).

Since hormones typically act on several physiological mechanisms simultaneously, they could thereby mediate suites of correlated behavioural traits (Ketterson & Nolan 1999). Shared common hormonal mechanisms could thus also explain the occurrence of behavioural syndromes (Bell 2007). Even though numerous studies have shown that differences in personality traits are in part genetically determined (reviewed in Van Oers et al. 2005; Penke et al. 2007), relatively few studies have explicitly investigated hormonal pathways of androgens by which consistent between-individual differences in exploratory behaviour, a key avian personality trait, might come about (but see King 2002).

In this study, we used house sparrows, *Passer domesticus*, to evaluate the relationship between exploratory behaviour (known to be part of a behavioural syndrome in birds) and circulating levels of T within and between individuals. We asked whether (experimentally induced) consistent individual variation in T affects exploratory behaviour (i.e. causes consistent individual variation in behaviour). We therefore quantified exploration of a novel environment in a captive population of male house sparrows. Such behaviour has frequently been used as a standard measure to quantify variation in a key 'avian personality' trait (see Dingemanse et al. 2002) and has been shown to correlate with numerous other behavioural traits, such as boldness, aggression and dominance (Dingemanse & De Goede 2004 and references therein).

First, we used observational data to test whether variation in exploratory behaviour correlated with two separate indexes of T levels: (1) a direct point estimate of circulating plasma T levels from a blood sample and (2) bill coloration. The degree of melanization of the bill has been shown to be a reliable method for estimating a recent 'running average' of circulating T levels (Keck 1933; Witschi 1936; Pfeiffer et al. 1944; Laucht et al. 2010), and is highly repeatable between consecutive years in the birds used for this study (Laucht et al. 2010). Second, we experimentally manipulated T levels of individual males with two distinct objectives. (1) We evaluated whether changes in behaviour within individuals were underpinned by changes in T levels, by comparing within-individual changes in behavioural traits before and after the T implantation. (2) We tested whether experimentally induced individual variation in T levels affected the amount of consistent individual variation in exploratory behaviour, by comparing a standardized index of variation between individuals (repeatability) with and without controlling for treatment effects.

METHODS

Animals and Housing

In December–January 2005–2006, about 1 year prior to the first experiments, we caught 136 male house sparrows with mist nets set up in barns around rural areas of Bavaria, Germany. The birds were transported to the institute by car, in dark compartments ($12 \times 12 \times 12$ cm) of a wooden box, and released into aviaries within 30–180 min of capture. They were housed in groups of 5–10 in adjacent semioutdoor aviaries (1.2×2.0 m and 4.0 m high) with one of the short sides enclosed only by chicken wire. All aviaries were fitted with two long perches crossing the aviaries (1.2 m), two wooden nestboxes, natural beech and spruce branches and sawdust. The birds were held under ambient outdoor temperatures and natural daylight conditions. They were fed ad libitum with a commercial seed mix ('Waldvogelfutter', RKW Süd, Universal Kraftfutterwerk, Kehl, Germany), sunflower seeds, crushed corn and wheat, pellets and laying mash and were given unrestricted access to drinking and bathing water and sand. We allowed the birds to breed every year, which they readily did. However, as the sex ratio was highly male biased (wild males were preferentially caught), not all males obtained a mate. The birds were caught and kept in captivity under licence from the government of Upper Bavaria. After we finished our experiments, the birds were kept for further studies.

Indexes of Male Testosterone Levels

Blood plasma testosterone

We collected 150–200 μl of blood from each individual by puncturing the brachial vein. All samples were obtained between 0900 and 1430 hours. To reduce variation in plasma T levels caused by variation in handling stress, we bled all individuals within 15 min after entering the housing aviaries for catching. Laucht et al. (2010) has shown for the same data set that the time between the start of catching birds and blood sampling does not affect plasma T levels, implying that effects of handling stress did not bias the data. After centrifuging the blood, we extracted the plasma and froze it at 80 °C. Plasma T levels were measured at the endocrine laboratory of the Institute for Zoo and Wildlife Research, Berlin, using the enzyme immunoassay described by Roelants et al. (2002). The interassay coefficient of variation (CV) was 12.3% and the intra-assay CV was 9.0%; for further details see Laucht et al. (2010).

Measured T levels were tested for cross-season repeatability as our observational study was conducted in the run-up to the breeding season (spring), whereas our experimental study was conducted in the nonbreeding season (autumn). Individual variation in spring plasma T levels was correlated, albeit weakly, with variation observed in the autumn (Pearson correlation: $r_{40} = 0.35$, $P = 0.02$; Appendix Fig. A1).

Bill coloration

In house sparrows, bill coloration varies from pale horn to jet-black and this variation can be quantified using 'brightness' measured on digitized photographs (Laucht et al. 2010). We took three standardized pictures of each bird the day before each behavioural trial. The focal bird was held laterally in front of a photographic grey card and photographed with a Canon Power Shot S2 IS using the flash and standardized settings. We used digital photograph processing software written in open source R version 2.8.0 (R Development Core Team 2008) to measure colour parameters on photographs. We measured grey card brightness (on the Hue, Saturation, Brightness colour scale) on each photo as the mean value of three different randomly chosen spots around the bill, and bill brightness as the mean of three different randomly chosen spots on the lower bill. We then averaged scores across the three photos. To compensate for slight variation in light conditions between pictures, we standardized bill brightness values with the mean brightness value of all the grey card brightness measures. Bill brightness was measured independently by two observers (A.M. and J.D.) and showed a high interobserver repeatability (Pearson correlation: $r_{134} = 0.95$, $P < 0.0001$). We used the mean brightness value of both scorers for further analysis.

Novel Environment Test

Following recommendations by Réale et al. (2007), we modified the standard novel environment test described by Verbeek

et al. (1994) for great tits, *Parus major*, so that it was suitable for house sparrows. The experimental room was an outdoor aviary (1.7 × 2.0 m and 3.5 m high) with side walls made of wire mesh and sackcloth, rear wall and ceiling made of wire mesh with a semitransparent plastic cover and the ground covered with sawdust and hay. The front consisted of a wooden wall, a one-way screen (45 × 45 cm) through which observations were made, and a sliding door through which the focal bird was released into the observation room. We equipped the aviary with nine objects, providing 10 different positions: an artificial tree, a nest-shaped bag on the wall, a nestbox (=two positions), a food bowl, a perch, a tunnel-shaped bag on the ceiling, a hanging tree, a mirror and a shelf (for a more detailed description see Appendix Fig. A2). We scored the position of the focal bird in the aviary for 30 min after introduction (using an event recorder; The Observer 5.0.31, Noldus Information Technology, Wageningen, The Netherlands). Based on these observations, we measured (1) exploratory behaviour, the total number of objects visited within 30 min, and (2) activity, the total number of hops (including only movements when the bird moved 10 cm or more) and flights within and between objects and positions. Exploratory behaviour did not correlate with activity (Spearman correlation: $r_S = -0.08$, $N = 114$, $P = 0.39$) and these two behavioural measures were therefore regarded as independent behavioural axes. We further recorded weather conditions and temperature for each trial.

Observational Study

In February–March 2007, 114 of the 136 captured birds were exposed to a first round of behavioural trials (between 0830 and 1600 hours). Blood samples for measuring T and pictures of the bill were collected for all the birds ($N = 136$) during the middle of the test period (early March 2007; Laucht et al. 2010). Each bird from a single housing aviary was captured with a hand net, weighed (±0.05 g measurement accuracy), and photographed on the afternoon before each round of behavioural trials, and then transferred to a cage (1.2 × 0.4 m and 0.4 m high) stocked with ad libitum food and water. The following day, we placed each focal bird in a darkened box (45 × 35 cm and 20 cm high) connected to the experimental room via a sliding door for a period of 5 min, enabling the bird to recover from handling stress. We started the trial by opening the sliding door and releasing the bird into the aviary. The focal bird was visually but not acoustically isolated from other group members throughout the trial.

Our observational study was conducted during the onset of the breeding season (in Germany, the average laying date for house sparrows is mid-April, but nest sites are already defended from January onwards; Glutz von Blotzheim 1997), because there is substantial individual variation in plasma T levels at this time of year (Kempenaers et al. 2008; Laucht et al. 2010), implying that any interindividual covariance with other traits (here: exploratory behaviour and activity) would also be estimable.

Approximately 9 months after the first round of trials (November–December 2007), we repeated the same trials described above with 48 randomly chosen (from the initial 114) birds to determine whether exploratory behaviour and activity each varied consistently between individuals over time, where repeatability was calculated following Lessells & Boag (1987).

Experimental Study

In November–December 2008, we performed another round of behavioural trials on a random subsample of 42 of the 114 previously tested birds. Half of the birds were assigned to a T treatment group, the other half to a control (C) group. Two weeks before the study, we separated the birds according to treatment and housed them in constant groups of five to seven birds throughout the experimental procedure. We implanted 21 birds subcutaneously with T pellets designed to imitate the upper range of plasma T levels during the breeding season in free-living house sparrows (approximately 5–6 ng/ml; Hegner & Wingfield 1986) with a release time of 21 days (Cat. No. A-151, 1.5 mg/pellet, IRA, Sarasota, Florida, U.S.A.). We implanted the other 21 birds with placebo pellets. We used the following implantation procedure. We caught all birds from one aviary to minimize disturbance and placed them individually in dark wooden boxes until the implantation took place. While holding the bird, we made a small incision (about 3 mm) in the upper layer of the skin on the back, inserted the pellet with tweezers and closed the incision with special glue (VetGlu, B.Braun Vet Care GmbH, Tuttlingen, Germany). Following implantation, birds were released immediately into their housing aviary. The maximum time between catching and releasing was 30 min. Our study was conducted in the nonbreeding season (autumn), because at this time of year plasma T levels are well below the peak values (Laucht et al. 2010), implying that experimental manipulation of plasma T levels would produce detectable increases within the natural range (i.e. ceiling effects might have occurred if the manipulation had been done during the breeding season instead).

Each of the 42 birds was behaviourally tested three times at intervals of 4 weeks using the same procedure as described above: once 3 weeks before implanting (trial 1), once 1 week after implanting (trial 2) and once 5 weeks after implanting (trial 3). In November–December 2008, we randomly chose three birds from the T treatment group and three birds from the C group within each test day, and assayed them in a random order to control for potential effects of time of day and temperature. We carried out the trials blindly, that is, without knowing to which treatment group the focal bird belonged. We took blood samples of every bird immediately after each of the three trials.

Statistics

Observational study

We used R version 2.8.0 (R Development Core Team 2008) for statistical analysis unless stated otherwise. T values were log transformed to achieve a normal distribution. Non-normally distributed data were Box–Cox transformed (number of objects, activity and bill brightness) and all data were standardized (i.e. expressed in standard deviation units) prior to analysis as recommended by Rasbash et al. (2004). We used general linear models (GLMs) to assess the relationship between the number of objects and activity and a number of response variables, where we used backward elimination of nonsignificant terms ($P \geq 0.05$) as a model selection criterion (Crawley 1993). We used the chi-square-distributed Wald statistic to calculate significance of predictor variables. The relationship between plasma T level and ornamentation measures and the relationship between T estimates and exploratory behaviour and activity were analysed with Pearson and Spearman rank correlations, respectively.

Experimental study

For statistical analysis of treatment effects, we used MLwiN version 2.0 (Rasbash et al. 2004). Data were Box–Cox transformed when necessary (number of objects and activity) to meet the distributional assumptions (normality) of multivariate linear mixed models (LMMs). T values were log transformed, and all data were standardized (expressed in standard deviation units) prior to analysis as recommended by Rasbash et al. (2004). A multivariate

LMM with individual as random effect and with normal errors was used to test simultaneously for manipulation effects on activity, exploratory behaviour, bill brightness and plasma T while taking into account the covariance between these four variables between and within individuals. Trial (before, during, after), treatment group (control versus T) and their interaction were fitted as fixed effects (all categorical variables). We used the chi-square-distributed Wald statistic to calculate significance of all fixed and random effects. Post hoc comparisons were performed to investigate further whether T treatment had a significant effect on plasma T levels. Repeatability was calculated from the multivariate LMM as the proportion of between-individual variation relative to the sum of the between- and within-individual variance for each trait (Rasbash et al. 2004) and standard errors were calculated following Becker (1984).

Ethical Note

Captive conditions

The birds used for this study were held in captivity for about a year prior to the study. We are confident that captivity did not have any adverse effect on the wellbeing of the birds because (1) the mean body mass of the captive birds (29.19 ± 1.72 g) was within the natural range for house sparrows in southern Germany (range 27–35.5 g, mean 29.9 g; Lowther & Cink 2006); (2) the annual mortality rate of our captive birds was between 0.05 and 0.09, which is much lower than mortality rates in free-living house sparrows (0.4–0.5; Senar & Copete 1995); and (3) our population readily breeds in the captive conditions provided, indicating conditions are generally favourable.

Testosterone implantation surgery

The implantation procedure took about 3 min per bird. The perforation of the rather loose upper layer of the skin did not cause bleeding. Moreover, none of the birds showed any sign of pain during the procedure (e.g. twitching) and all birds started feeding within 15 min after release (A. Mutzel, personal observation). The procedure applied is nearly identical to the implantation of passive integrated transponder (PIT) tags. Fitting birds with PIT tags has been shown to have no adverse effect on the survival and recruitment of nestling and adult great tits (Nicolaus et al. 2008). We did not use anaesthesia as this can have harmful effects on birds, including a high risk of death (Ludders 1998). In this study, experimental treatment did not appear to have any long-term adverse effect on the birds, because the mortality rate of the experimental birds in 2009 was slightly lower than that of nonexperimental birds (0.07 versus 0.09). The implantation was approved by the government of Upper Bavaria and carried out in accordance with the German animal protection law.

Table 1
Sources of variation in exploratory behaviour and activity in male house sparrows ($N = 114$)

	Exploratory behaviour		Activity	
	Wald χ^2_1	P	Wald χ^2_1	P
Date	0.02	0.89	0.18	0.68
Time of day	11.69	**<0.001**	0.83	0.36
Weather condition	2.68	0.10	8.52	**0.03**
Temperature	0.25	0.62	4.84	**0.03**
Bird mass	0.53	0.47	5.18	**0.02**

The results are from univariate GLMs with exploratory behaviour or activity as response variable, and date, time of day (in minutes after sunrise), weather conditions (sun/clouds/precipitation), temperature (in °C) and bird mass fitted as predictor variables. We used backward elimination of nonsignificant terms; Wald chi-square values given are for the inclusion of the variable in the final model. Significant P values are indicated in bold.

Table 2
Spearman rank correlations for exploratory behaviour, and activity, with circulating plasma T level and bill brightness (bill) in male house sparrows ($N = 114$)

	Exploratory behaviour		Activity	
	r_S	P	r_S	P
Plasma T	−0.02	0.81	0.04	0.67
Bill	0.00	0.97	−0.04	0.64

RESULTS

Observational Study

As expected, bill brightness at the time of blood sampling was negatively correlated with plasma T levels (Pearson correlation: $r_{134} = 0.25$, $P = 0.003$): birds with a paler bill had on average lower plasma T levels than birds with darker bills. Exploratory behaviour was significantly affected by time of day for first tests (Table 1). Date, temperature, weather conditions and body mass did not correlate with exploratory behaviour, and were therefore not included in the final model. Plasma T levels and bill brightness on

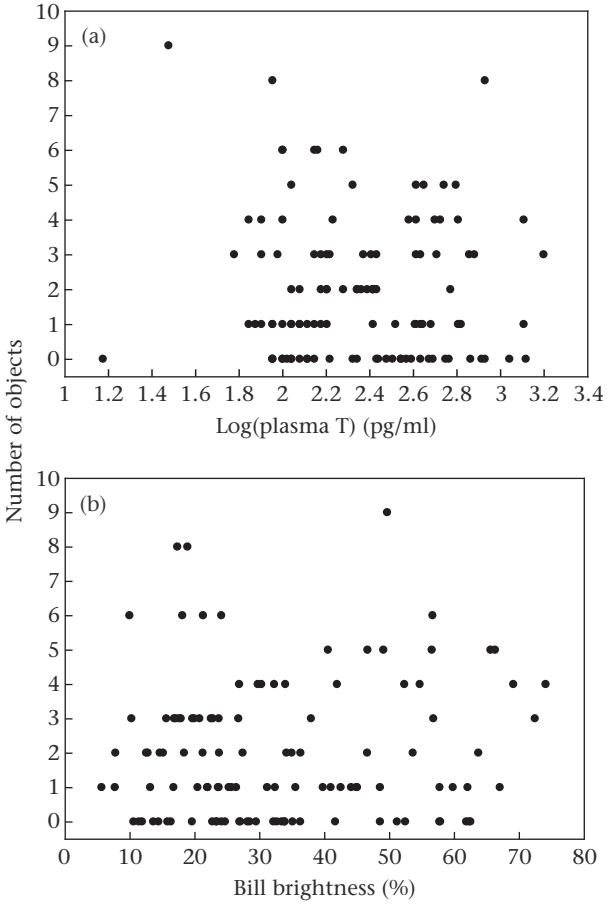

Figure 1. Correlation between exploratory behaviour (actual number of objects explored within 30 min) and two estimates of individual T levels in male house sparrows. (a) Plasma T level ($r_S = -0.02$) and (b) bill brightness ($r_S = 0.00$) as a percentage.

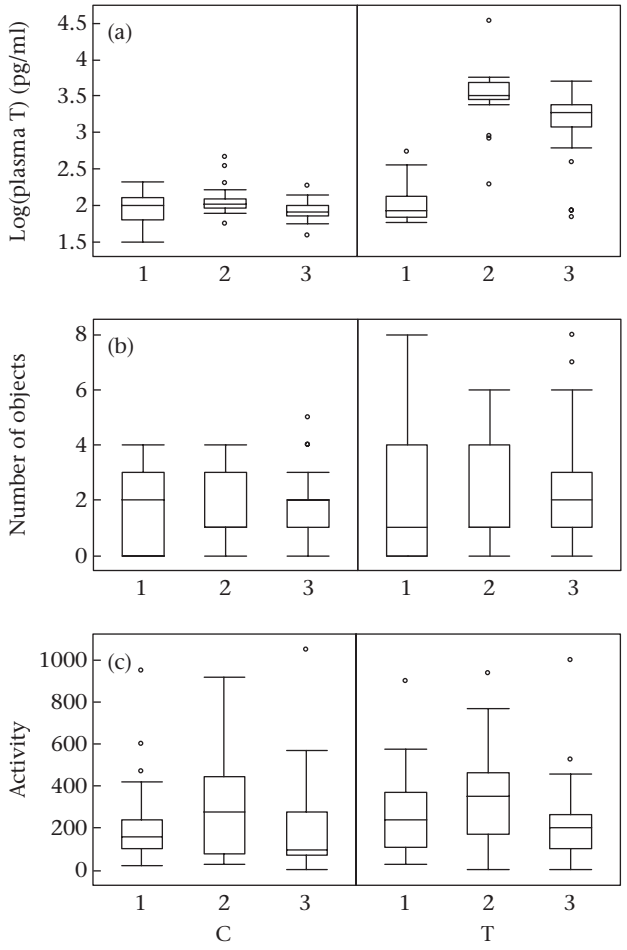

Figure 2. Plasma T levels and behaviour in the control (C) group and the testosterone (T) treatment group before (trial 1), 1 week after (trial 2) and 5 weeks after (trial 3) the implantation in male house sparrows. (a) Measured plasma T levels, (b) exploratory behaviour (actual number of objects visited) and (c) activity (total number of hops and flights). Box plots show the median and the interquartile range from the 25th to the 75th percentile. Whiskers indicate the 10th to the 90th percentile. Dots show outliers.

the day before the behavioural assay did not correlate significantly with exploratory behaviour (Table 2, Fig. 1). Activity was influenced by temperature, weather conditions and body mass before the experiment, but not by date or time of day (Table 1). Activity levels were higher on relatively rainy and cool days. Heavier birds were also more active than relatively light birds. Neither plasma T nor bill brightness was correlated with activity (Table 2). The behaviour in a novel environment varied consistently between the 48 individuals that were retested 9 months after their initial test. Repeatability was 0.45 ± 0.12 ($F_{47,48} = 2.62$, $P < 0.001$) for exploratory behaviour and 0.48 ± 0.11 ($F_{47,48} = 2.82, P < 0.001$) for activity.

Experimental Study

To investigate whether T treatment affected plasma T levels and bill brightness, we examined the interaction between trial and treatment group (the actual T treatment took place during trial 2) and changes in these traits with trial. As expected, we found highly significant effects of T implantation on plasma T levels (interaction trial*treatment: $\chi^2_2 = 117.87$, $P < 0.001$) and bill brightness (interaction trial*treatment: $\chi^2_2 = 70.85$, $P < 0.001$), implying that within-individual variation in T causally influenced the expression of these phenotypic traits. To explore the nature of the treatment effect, we performed post hoc comparisons. Within the C group, plasma T levels did not differ between trials (Fig. 2a, Appendix Table A1). Within the T treatment group, we found a significant effect of trial on plasma T levels. The highest levels were recorded directly following T manipulation (trial 2) and were still elevated in trial 3 compared to T levels at the beginning of the experiment (Fig. 2a, Appendix Table A1). Furthermore, plasma T levels differed significantly between the C and T groups after the experimental manipulation (in trials 2 and 3) but not before, implying that we succeeded in our aim to induce between-individual variation in T levels (see also Table 3). Birds from the T treatment group showed significantly higher plasma T levels than individuals from the C group ($P < 0.001$; Fig. 2a, Appendix Table A1). Plasma T levels of the birds from the C group ranged between 0.03 and 0.45 ng/ml (mean 0.11 ± 0.06 ng/ml). In the T treatment group plasma T levels were 0.06–0.55 ng/ml (mean 0.13 ± 0.12 ng/ml) in trial 1, 0.19–33.66 ng/ml (mean 4.72 ± 6.79 ng/ml) in trial 2 and 0.07–5.07 ng/ml (mean 1.92 ± 1.39 ng/ml) in trial 3. Excluding the unusually high plasma T value of one bird in trial 2 (33.66 ng/ml) did not change the results of the study.

To investigate whether T treatment had an effect on exploratory behaviour and activity, we examined the interaction of trial and treatment group (see above). Contrary to our prediction, neither exploratory behaviour ($\chi^2_2 = 0.35$, $P = 0.84$; Fig. 2b) nor activity ($\chi^2_2 = 0.3$, $P = 0.86$; Fig. 2c) was affected by T treatment. Next, we ran univariate LMMs to assess whether T treatment relates to individual differences in behaviour. Even though activity and exploratory behaviour were both highly repeatable, there was no significant change in repeatability of these behaviours before and after controlling for trial, treatment group and their interaction

Table 3
Variance components, repeatability values and significance for assayed phenotypic traits

	Controlling for fixed effects?	Variance component±SE		Repeatability		
		Between-individual variance	Within-individual variance	r±SE	χ^2_1	P
Activity	Yes	0.53±0.15	0.42±0.06	0.56±0.08	37.78	**<0.001**
	No	0.53±0.15	0.47±0.07	0.53±0.09	32.93	**<0.001**
Exploration	Yes	0.64±0.17	0.34±0.05	0.66±0.07	54.58	**<0.001**
	No	0.65±0.17	0.35±0.05	0.65±0.07	53.44	**<0.001**
Plasma T	Yes	0.03±0.02	0.18±0.03	0.14±0.10	2.31	0.129
	No	0.22±0.11	0.77±0.12	0.22±0.10	5.57	**0.018**
Bill	Yes	0.33±0.09	0.28±0.04	0.55±0.09	35.62	**<0.001**
	No	0.46±0.14	0.54±0.08	0.46±0.09	24.47	**<0.001**

Results are from univariate general mixed models with individual as random effect and activity, exploratory behaviour, plasma T level and bill brightness (bill) as dependent variables ($N = 42$ male house sparrows). In models where we controlled for fixed effects, the fixed effects included in the model are trial (before, during, after), treatment group (T and C) and the interaction between trial and treatment group. Significant P values are indicated in bold.

(Table 3): only 1% of the observed between-individual variance in activity and 0.1% of the variance in number of objects examined could be attributed to trial, treatment group and their interaction. These findings thus confirm experimentally that between-individual variation in T levels is not linked to between-individual variation in exploratory behaviour.

DISCUSSION

T Levels and Exploratory Behaviour

This study showed that individual differences exist in exploratory behaviour and activity in male house sparrows, but that these differences cannot be attributed to variation in circulating levels of the hormone T. Plasma T titre was not correlated with these two personality traits within our observational data set. Moreover, within individuals, experimentally elevated plasma T levels caused no changes (i.e. within-individual plasticity) in either exploratory behaviour or activity. Finally, experimentally induced differences in T levels did not affect the amount of individual variation in these behaviours.

In agreement with these findings, we did not detect any significant relationship between a reliable short-term proxy of T level (bill brightness) and the two personality traits. Importantly, we confirmed that bill brightness correlated significantly with circulating plasma T levels in our descriptive data set, and that implantation with T caused the bill of male sparrows to darken considerably (Witschi 1936; Pfeiffer et al. 1944). Bill coloration thus reliably predicts a short-term (i.e. 3–4 weeks) 'running average' of T levels. Our failure to find associations between this proxy for T and the two behaviours underlines the absence of T-mediated variation in exploratory behaviour.

Few other studies have investigated the relationship between T and consistent between-individual differences in behavioural traits. In agreement with our results, an observational study by Sellers et al. (2007) on humans also failed to detect any correlation between levels of circulating T and openness to experience, a human personality trait considered to be the equivalent of exploration behaviour in nonhuman animals (Gosling & John 1999). Similarly, in male greylag geese, *Anser anser*, T did not covary with another component of avian personality (aggressiveness; Kralj-Fišer et al. 2010). The results from other studies imply that the behavioural effects of circulating levels of T are either inconsistent or context specific. Some studies on domesticated birds have provided evidence for a causal effect of circulating plasma T levels on certain behavioural traits. For instance, domestic chickens, *Gallus gallus domesticus*, with experimentally increased circulating levels of T showed increased persistence in search for a particular type of food and pecked more often on one particular square before moving on to the next square (Andrew 1972; Rogers 1974; Young & Rogers 1978). However, in another study on domestic chickens, Archer (1973) failed to detect an effect of T on the number of squares explored during a novel environment test in a nonforaging context. However, T-treated males showed shorter latencies to move in a novel environment than controls. In contrast, King (2002) found no relationship between T and the latency to move after transfer to a novel environment in garter snakes, *Thamnophis sirtalis*, but showed instead that defensive behaviour was influenced by circulating levels of T. Furthermore, Jones & Andrew (1992) found T-related differences between male and female domestic chickens in the response to novelty. However, this effect seemed to be context dependent, with males being more explorative in a novel environment but less explorative in a foraging context. Other studies found that T is only related to dominance and aggression when dominance status is uncertain but not in socially stable situations (Ruiz-de-la-Torre & Manteca 1999; Collias et al. 2002). The potential effects of T on exploration and activity might therefore not be general, but rather depend on the context in which the behaviour is shown. For instance, it is possible that T mediates the expression of certain behaviours within a social context, but that it is less important in nonsocial situations, for example in a novel environment. This context dependency of the link between T and behaviour has recently been suggested by Koolhaas et al. (2010) for the relationship between T and aggression. To test this idea, it would be interesting to investigate the relationship between circulating plasma T levels and a number of different personality traits while manipulating context (i.e. social versus nonsocial).

Cross-season Repeatability of T

In many vertebrate species, plasma T levels show pronounced seasonal variation within individuals, with peak values occurring during the breeding season (Kempenaers et al. 2008). Whereas such seasonal changes in T levels have received considerable attention at the within-individual level, fairly little is known about (1) patterns of individual variation (e.g. repeatability) in plasma levels, (2) whether individual variation exists only in certain seasons, and (3) whether any differences between individuals in plasma T levels are maintained across seasons (i.e. is there cross-season repeatability?; Kempenaers et al. 2008; Laucht et al. 2010). Such information is valuable because it would facilitate further interpretation of our findings, as our study was carried out in two different seasons. We discuss here two scenarios. First, there is no cross-season repeatability of plasma T. This scenario would imply any individual variation observed within a season is not proximately linked with variation observed in other seasons (i.e. spring and autumn levels of T should be regarded as different 'traits') and that we tested different questions with our observational and experimental studies. Additionally, our experimental study could not be used to test whether any link between plasma T and personality traits documented in our observational study was causal. Second, there is cross-season repeatability of plasma T. This scenario would imply that individual variation observed within a season is proximately linked with variation observed in other seasons (i.e. spring and autumn levels of T measured the same trait). We tested for a cross-season repeatability of plasma T and found that there was a statistically significant but low (Pearson: $r = 0.35$) correlation. This result implies that our experimental study can be used to evaluate the causal link between plasma T and exploratory behaviour observed in other seasons (however, also see Laucht et al. 2010).

Alternative Pathways by which T could Affect Personality

Although our observational study did not provide any supportive evidence in favour of a relationship between circulating plasma T levels and exploratory behaviour and activity, there are several alternative pathways by which T could still mediate between-individual differences in these personality traits (Ketterson & Nolan 1992). For instance, there might be genetically determined variation in T receptor density, affinity and specificity that does not necessarily have to be linked with circulating levels of T (Adkins-Regan 2005; Ball & Balthazart 2008). Moreover, it is known that heritable variation in plasma-binding proteins can have substantial effects on the availability of a certain hormone (Dufty et al. 2002; Ball & Balthazart 2008). Potential effects of T on behaviour acting via between-individual differences in such alternative pathways might simply be obscured when one measures

circulating plasma T levels only. However, under such scenarios we would expect changes in behaviour following experimental elevations of T, which we did not find. Therefore it seems rather unlikely that such alternative hormonal mechanisms would affect variation in exploratory behaviour.

Nevertheless, we cannot completely exclude the possibility of a link between T and exploratory behaviour. For instance, phenotypic expression of T receptors could match phenotypic expression of circulating hormone levels. In this case endogenous levels of T might already be sufficient to saturate the receptors and experimentally increasing T levels would not have any additional effect on behaviour. It might also be that T receptor sensitivity is lower in the nonbreeding season than in the breeding season (Canoine et al. 2007). In this case, we do not expect to find a strong effect of T treatment on behaviour. However, this scenario is rather unlikely, because T treatment had a strong effect on bill coloration, a trait that has been suggested to be a good indicator of quality and/or behavioural strategies (Laucht et al. 2010).

T could also (indirectly) affect behaviour through an alternative pathway involving the stress hormone corticosterone. There is good evidence that corticosterone responses are linked with certain behavioural types (reviewed in Cockrem 2007). Moreover, numerous studies have shown that corticosterone and T titres tend to covary (e.g. Wilson et al. 1979; Siegel 1980; but see also Hau et al. 2010). Therefore, observed links between T and behaviour in certain contexts may be caused by a direct effect of T on the behaviour or an indirect effect via corticosterone. More studies are needed to investigate potential direct and indirect links between corticosterone and T and behaviour.

This study did not provide any evidence in support of a relationship between circulating plasma T levels and exploratory behaviour. We should underscore that we focused here on activational effects of T on behaviour. T could still be involved in forming this personality trait through its organizational effects, that is, by causing irreversible differences in behavioural phenotype during early development of an individual (Daisley et al. 2005; Groothuis et al. 2005). Therefore, another fruitful next step would be to investigate the relationship between maternal T deposited in eggs and personality traits.

Conclusions

We showed that male house sparrows differ consistently in exploratory behaviour and activity in a novel environment. This variation was not related to circulating levels of plasma T. Our results thus challenge the assumption of a T-based mechanism causing variation in these personality traits. Furthermore, our results suggest that T is not part of potential proximate mechanisms that cause behavioural syndromes, which include exploratory behaviour or activity. However, T might still be involved in creating individual differences in other personality traits, such as boldness or aggressiveness or influence activity and exploration within other, for example social, contexts.

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APPENDIX

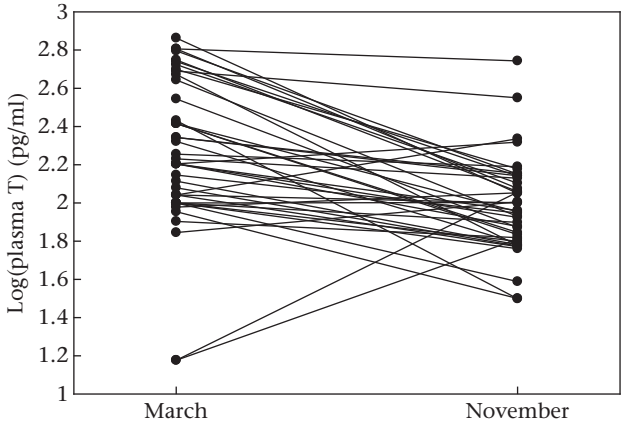

Figure A1. Plasma T levels in March and November 2007 of the individuals used for the experimental study ($N = 42$). The black lines connect plasma T measurements of the same individual at the two seasons. Parallel lines indicate cross-season repeatability of T levels.

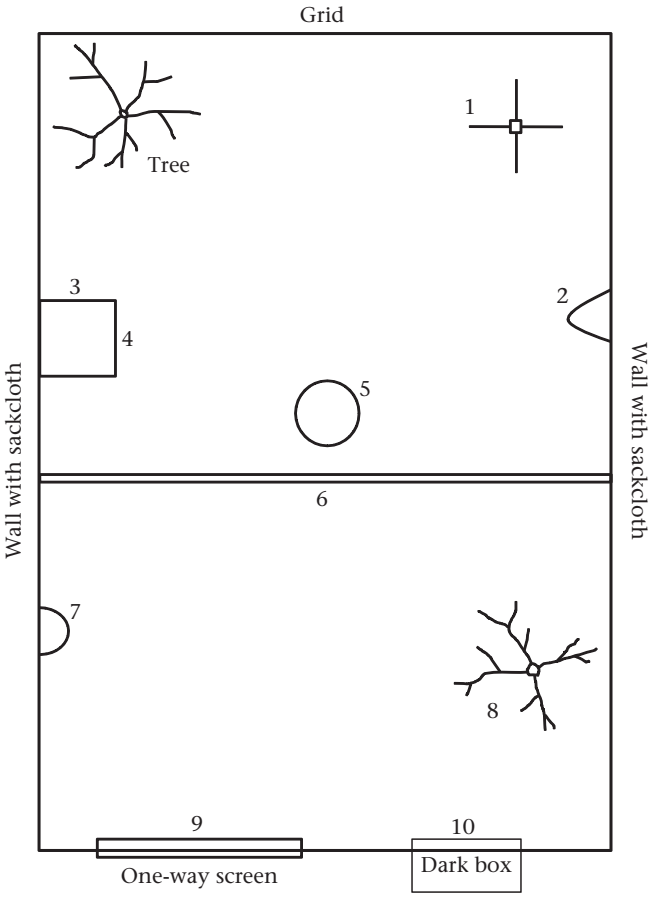

Figure A2. Schematic overview of the observation room. (1) Artificial tree (made of wood with a trunk of 4 × 4 cm, height 1.5 m and four cylindrical branches of 20 cm), (2) bag on wall (nest shaped, made of sackcloth), (3) + (4) nestbox (roof and inside), (5) food bowl with five sunflower seeds (on the ground, diameter 30 cm), (6) perch (crossing the aviary, length 1.7 m), (7) bag on ceiling (tunnel shaped, made of sackcloth), (8) hanging tree (branch hanging from the ceiling, 50 cm in length), (9) mirror (one-way screen through which observations were made), (10) shelf (in front of the sliding door).

Table A1
Results from a post hoc comparison of a linear mixed model with individual as random effect and trial, treatment group and their interaction as fixed effects

	χ^2	*df*	*P*	β±SE
Control group				
Trial 2 vs 1				
Activity	3.058	1	0.080	0.348±0.199
No. of objects	0.054	1	0.816	0.042±0.179
Bill brightness	6.129	1	**0.013**	0.401±0.162
Plasma T	1.608	1	0.205	0.168±0.033
Joint	10.43	4	**0.034**	
Trial 3 vs 1				
Activity	0.358	1	0.550	−0.119±0.199
No. of objects	0.464	1	0.496	0.122±0.179
Bill brightness	15.49	1	**<0.001**	0.637±0.162
Plasma T	0.025	1	0.874	−0.021±0.133
Joint	17.42	4	**0.002**	
Trial 3 vs 2				
Activity	5.508	1	**0.019**	−0.466±0.199
No. of objects	0.201	1	0.654	0.080±0.179
Bill brightness	2.133	1	0.144	0.236±0.162
Plasma T	2.035	1	0.154	−0.189±0.133
Joint	11.77	4	**0.019**	
Testosterone treatment group				
Trial 2 vs 1				
Activity	1.011	1	0.315	0.200±0.199
No. of objects	0.830	1	0.362	0.163±0.179
Bill brightness	14.11	1	**<0.001**	−0.608±0.162
Plasma T	248.7	1	**<0.001**	2.090±0.133
Joint	255.2	4	**<0.001**	
Trial 3 vs 1				
Activity	1.342	1	0.247	−0.230±0.199
No. of objects	2.095	1	0.148	0.259±0.179
Bill brightness	63.41	1	**<0.001**	1.289±0.162
Plasma T	131.4	1	**<0.001**	1.519±0.133
Joint	186.5	4	**<0.001**	
Trial 3 vs 2				
Activity	4.683	1	**0.030**	−0.430±0.199
No. of objects	0.288	1	0.592	0.096±0.179
Bill brightness	17.70	1	**<0.001**	−0.681±0.162
Plasma T	18.55	1	**<0.001**	−0.571±0.133
Joint	44.34	4	**<0.001**	
Within trials				
Trial 1				
Activity	0.805	1	0.370	0.278±0.300
No. of objects	0.083	1	0.773	0.088±0.305
Bill brightness	0.062	1	0.803	0.060±0.241
Plasma T	0.367	1	0.545	0.087±0.143
Joint	1.602	4	0.808	
Trial 2				
Activity	0.187	1	0.665	0.130±0.300
No. of objects	0.470	1	0.493	0.209±0.305
Bill brightness	15.52	1	**<0.001**	−0.948±0.241
Plasma T	197.5	1	**<0.001**	2.009±0.143
Joint	212.4	4	**<0.001**	
Trial 3				
Activity	0.307	1	0.580	0.166±0.300
No. of objects	0.544	1	0.461	0.225±0.305
Bill brightness	60.07	1	**<0.001**	−1.865±0.241
Plasma T	129.5	1	**<0.001**	1.627±0.143
Joint	185.2	4	**<0.001**	

Trial differences within the control group, trial differences within the testosterone treatment group and treatment group differences within trials are shown. Significant *P* values are indicated in bold.

Chapter 2

Temporal trade-offs between nestling provisioning and defence against nest predators in blue tits

Ariane Mutzel, Mozes P.K. Blom, Foteini Spagopoulou, Jonathan Wright, Niels J. Dingemanse & Bart Kempenaers

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Temporal trade-offs between nestling provisioning and defence against nest predators in blue tits☆

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Offspring provisioning and nest defence are important forms of parental care. In birds, parents that engage in nest defence behaviour have to interrupt nestling provisioning with potentially harmful consequences for offspring growth and condition. To maximize fitness, parents should trade off optimal levels of offspring provisioning versus nest defence, but relatively little is known about how parents allocate their time between these two activities and how parental decisions to postpone provisioning vary as a function of the intensity of nest predation risk. We found that pairs of blue tits, *Cyanistes caeruleus*, adjusted parental care behaviours according to perceived immediate risk levels by switching from offspring provisioning to nest defence. In the presence of a direct nest predation threat, parents interrupted offspring provisioning for longer than in response to a novel object close to the nest, but still gradually resumed provisioning activity, probably because of a decrease in perceived predation risk over time. By increasing their provisioning effort once the immediate threat had diminished, parents compensated at least partly for the lost provisioning opportunities during high-risk situations. Hence, by adaptively adjusting the temporal trade-off between different parental care behaviours according to the perceived risk, blue tits are presumably able to mitigate potential negative long-term consequences of interruptions in provisioning during high-risk situations for offspring growth and condition.

Offspring provisioning systems have become a model for the behavioural study of adaptive parental investment (Clutton-Brock 1991; Royle et al. 2012), biparental cooperation (Houston et al. 2005; Harrison et al. 2009), and parent–offspring conflict and the evolution of begging signals (Wright & Leonard 2002; Hinde & Kilner 2007; Smiseth et al. 2008). However, other important aspects of parental care, such as antipredator defence of offspring, are rarely studied alongside offspring provisioning. This is surprising, because these activities should trade off with, or interrupt, provisioning because of limitations in time and energy. Such trade-offs could be critical for adaptive levels of provisioning effort and optimal behavioural responses to changes in conditions (Markman et al. 1995, 1996; Rauter & Moore 2004).

In birds, predation is an important cause of nest failure (Ricklefs 1969), and birds are able to minimize nest predation risk through plastic behavioural responses towards the actual threat level (Lima 2009; Martin & Briskie 2009). Besides nestling provisioning, nest defence is an important form of avian parental care (Shields 1984), which can range from vocal mobbing from a safe distance to physically attacking the predator, and is assumed to have evolved to reduce losses of nestlings to nest predators (Montgomerie & Weatherhead 1988). This assumption is confirmed by numerous studies, showing a positive relationship between nest defence behaviour and nest success (e.g. Greig-Smith 1980; Blancher & Robertson 1982; Markman et al. 1996).

Even though antipredator behaviour might be beneficial in terms of immediate nestling survival, it also incurs costs for parents and offspring that might depend upon the type of nest predator

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(Lima 2009). For example, nest defence against predators that also prey upon adult birds would represent a relatively high potential fitness cost, because of the additional risk of adult predation. In contrast, the presence of a nest predator that only consumes nestlings represents little direct risk to the parents, but defence might be costly in terms of offspring production because of harmful interruptions to nestling provisioning. Parents therefore face the dilemma of trading off the two mutually exclusive behaviours of offspring provisioning and nest defence. Doing so allows them to maximize the productivity of the current nesting attempt while taking into account effects on their future reproductive output (i.e. the parent's ability to invest in future broods: Trivers 1972). One way that parents could do this is to adjust provisioning activity over time according to the variation in nest predation risk.

Despite the large number of studies on immediate antipredator nest defence or mobbing, relatively little is known about the temporal patterns of alternative parental care behaviours in different risk situations. For instance, perceived predation risk is expected to differ before, during and after the encounter with a nest predator (Tilgar et al. 2011). Even during the presence of a predator, the response of the parents should diminish over time owing to adaptive habituation (Rankin et al. 2009). Hence, to minimize both the costs of potential nest predation and nestling starvation, parents should allocate different amounts of time to provisioning and nest defence according to the risks involved (Lima 2009). During high predation risk situations, parents should reduce nest visit rates and/or engage in vigorous nest defence behaviour, whereas parents should maintain nestling provisioning at a rate closer to normal levels when nest predation risk is relatively low (Martin & Briskie 2009). This expectation is confirmed by a number of empirical studies showing that increased risk of predation on dependent offspring can cause parents to reduce their provisioning rate temporarily, if only to reduce the chances of nest detection by the predator (e.g. Ghalambor & Martin 2000, 2001; Eggers et al. 2005; Fontaine & Martin 2006; Peluc et al. 2008). However, to understand the temporal trade-off between provisioning and nest defence, we also need to know how the perceived predation threat changes over time, that is, how quickly parents habituate to the threat and resume provisioning the young in the nest.

The temporal trade-off between nest defence and offspring provisioning will also depend upon the underlying mechanisms of predator recognition and the individual discrimination abilities needed to produce adaptive differences in parental responses to different types of predation threat. Predator models have been shown to elicit antipredator responses similar to those of live predators (Curio 1975), and there has been a long history of investigating (functional) differences in parental responses to different types of model predators (Lima 2009; Martin & Briskie 2009). In contrast, engaging in extended nest defence behaviour against a harmless (novel) object is expected to be nonadaptive as it does not increase nest survival, but nevertheless incurs costs in terms of lost provisioning opportunities. To be able to show adaptive behavioural responses to nest predation threats, parents need to differentiate correctly between different levels of threat represented by known nest predation threats versus novel but harmless situations, and to adjust the scale of their response and its decline over time if the situation persists. In this study, we aimed to investigate whether actual predator recognition is involved in driving such parental responses, that is, whether parents behave differently towards a taxidermic model of a real nest predator or whether they merely respond to new objects around the nest (Curio 1975).

We investigated the responses of provisioning pairs of blue tits, *Cyanistes caeruleus*, towards a taxidermic model of a great spotted woodpecker, *Dendrocopos major*. This species is a typical nest predator of Eurasian cavity-nesting birds (Löhrl 1972) and can inflict heavy losses on nests (Curio & Onnebrink 1995). In contrast, it represents little mortality risk to adult birds (Curio 1975). The typical nest defence behaviour of great tits, *Parus major*, and blue tits towards the presence of a woodpecker close to their nest consists of extended vocal mobbing (largely 'churr' calls) combined with frequent movements between perches, thereby rarely approaching the predator closely (Onnebrink & Curio 1991). Parental alarm calls might distract predators away from the vulnerable offspring and/or might warn nestlings about the predation risk (Harvey & Greenwood 1978; Greig-Smith 1980). We investigated in detail the temporal trade-off between nest defence and provisioning behaviour in blue tit pairs before, during and after an encounter with a model of this potential nest predator. We also explored whether blue tits are able to recognize a potential nest predator by comparing parental responses to a model predator and to a novel but nonthreatening object (a red rubber ball).

METHODS

Study Site and General Field Procedures

The study was carried out during the breeding season of 2011 on a nestbox population of blue tits in southern Germany (Westerholz, 48°08′N, 10°53′E). The Westerholz forest mainly consists of mature oak trees, *Quercus* sp. (for more details see Schlicht et al. 2012), the preferred habitat of blue tits (Gibb 1954). Adults were caught inside the nestbox during nestling provisioning when nestlings were 9 or 10 days old. Unbanded birds were fitted with a numbered metal band and a unique combination of three colour bands and equipped with a uniquely coded passive integrated transponder (PIT) tag (EM4102 ISO animal tag 134.2 kHz ISO, 8.5 mm × 2.12 mm, 0.067 g) following procedures detailed by Nicolaus et al. (2008). All nestlings were banded and measured when they were 14 days old.

Automated Recording of Provisioning Behaviour

Provisioning behaviour of the parents was recorded on 4 consecutive days at 48 nestboxes with automatic nestbox recording devices when nestlings were between 11 and 14 days old. For nestboxes where nestlings were processed less than 2 h before the planned data recording, we collected data on day 15 instead ($N = 14$ nestboxes), to ensure that nestling processing at day 14 did not affect our measures of control feeding rate. The recording device consisted of an antenna around the entrance hole (PIT tag reader), one light barrier inside and one outside next to the nestbox hole, a power supply and a data logger placed on the ground underneath the nestbox (for technical details see Schlicht et al. 2012). The sequence of activation of the two light barriers indicated the direction of the movement of a bird, allowing differentiation of entries and exits. Every time the bird passed through the nestbox hole the PIT tag was read, thus determining the identity of the bird entering or leaving the nestbox. However, owing to variation in sunlight reflection, light barriers sometimes did not work properly. Consequently, we checked all recorded data files to determine entry and exit times of PIT-tagged birds. We excluded trials (for a definition of trial see below) with unreliable data (i.e. with more than one nonassigned visit) from the final data set. In total, 33 of 288 control trials, 13 of 144 red ball trials and eight of 147 woodpecker trials were excluded from the analysis, resulting in a total sample size of 2596 visits for control day 1, 2826 for the red ball treatment day, 2394 for the woodpecker treatment day and 2609 for control day 2. We used nest visit rate as a proxy for feeding rate, as there is little variation in prey sizes and nonfeeding visits are rare at this stage in the nestling period (Kluijver 1950; Eguchi 1980; Nur 1984). Video recordings from 52 nests further showed prey delivery

even at the first visit after the experimental presentation of woodpecker models and red balls near the nest, as described below (unpublished data from the same population).

Perceived Predation Threat Experiment

We tested the response of pairs of blue tits towards potential nest predators when nestlings were either 12 (group 1) or 13 (group 2) days old (Table 1). We did this by presenting a taxidermic model of a great spotted woodpecker (WP) fixed on a wooden, 1.5 m high pole 2 m from the target nestbox with the woodpecker's bill facing the entrance hole. Each experiment included three consecutive 30 min trials: a pretreatment 'control' (trial 1) preceding the experimental treatment (trial 2) and a post-treatment 'control' (trial 3; Table 1). We conducted the same test procedure with a red rubber massage ball of 8 cm diameter ('red ball', RB) instead of the woodpecker model on day 13 (group 1) or day 12 (group 2; Table 1). The red ball treatment was performed to distinguish between effects provoked by fear of novelty (i.e. changes in the immediate nest environment) from effects caused by the presence of a potential nest predator. Given the identical nature of these manufactured objects, there was no need to use multiple red ball models (as we did with the woodpecker models).

Each nest was randomly assigned to a group in a stratified manner such that half of the nests were assigned to group 1 and 2, respectively. We did this by assigning the first nest randomly to one of the groups, the subsequent nest to the alternative group and then continuing assignment in an alternate order over the days of study. Later analysis revealed that there was no significant effect of test sequence (Appendix Table A1) confirming that the latter did not bias treatment effects. We used nine different woodpecker models that were randomly assigned to the experiments to avoid pseudoreplication (Hurlbert 1984). Woodpecker identity did not significantly affect parental responses (see Appendix), and was therefore not included in subsequent analyses. Via a quick visual and acoustic inspection, we also noted whether one or both parents were present around the nest at the onset and the end of each experimental trial (trial 2) and whether they engaged in nest defence behaviour. In addition to the two experimental (WP and RB) treatments, we also collected 'control data' the day before and the day after the two experiments (Table 1) following the same protocol and at the same time of day. This allowed us to check for any carryover effects of the experiments, over and above any changes that were attributable to simple time-of-day or nestling age effects. Provisioning data of the control treatment days (control 1 and 2; Table 1) were combined into a single control treatment. We also ran all analyses with the two separate control treatments (i.e. with four treatment groups), but this did not change any of the conclusions. We therefore only present results of the simplified analyses. In contrast to the 2 experimental days, where the observer approached the nestbox at the onset and at the end of trial 2 (to install and remove the object), there was no human disturbance during control days.

Table 1
Overview of the experimental set-up of the study

Experimental day	Brood age (days)	Treatment groups		Treatment	Trial type	Object present
1	11	Both groups:	Control 1	C	1	No
					2	No
					3	No
2	12	Group 1:	Woodpecker	WP/RB	1	No
		Group 2:	Red ball		2	Yes
					3	No
3	13	Group 1:	Red ball	RB/WP	1	No
		Group 2:	Woodpecker		2	Yes
					3	No
4	14	Both groups:	Control 2	C	1	No
					2	No
					3	No

The red ball (RB) and the woodpecker (WP) model treatments were both carried out on all nests in a random order on consecutive days. Nestboxes assigned to group 1 received the WP treatment first when broods were 12 days old and then RB treatment when broods were 13 days old, with this being reversed for group 2. Control treatments took place 1 day before (brood age 11) and 1 day after (brood age 14) the experimental treatments for both groups. At some nestboxes provisioning data were not available for brood age day 14 and were therefore collected on day 15.

Behavioural Responses

From the automatically collected PIT tag data, we quantified: (1) the initial 'feeding latency' at the start of each trial, measured as the time elapsed between the start of the trial and the focal individual's first entrance into the nestbox (feeding latency exceeded the 30 min trial duration for individuals that completely interrupted feeding during a trial, see Results); and (2) the temporal pattern of provisioning by extracting the 'intervisit interval' (IVI; the time elapsed between the exit of the previous visit and the entrance of the focal visit for each individual; see Wright et al. 2010). Note that the first IVI per bird for each trial used the last exit of the previous trial, and so contained some variation because of variation in feeding latency (see above). For cases in which an individual completely interrupted feeding during the previous trial (which was the case for some individuals during the WP and RB presentation, trial 2), we used the last exit of this individual at the trial before (i.e. trial 1), resulting in IVIs exceeding 1800 s. Temporal patterns in IVIs were used to explore to what extent the presentation of a predator model had a negative effect on parental provisioning early on in the trial over and above the initial latency (Ghalambor & Martin 2000; Peluc et al. 2008). They were also used to reveal any compensatory increases in parental provisioning later on in these trials in response to increased begging of hungry nestlings. (3) From the behavioural observations at the onset and the end of the model presentation, we quantified whether one or both parents engaged in vocal mobbing behaviour, hereafter referred to as nest defence.

Statistical Analysis

Feeding latency

We analysed sources of within-individual variation in feeding latencies using linear mixed-effect models with brood age, sex, trial type and treatment fitted as fixed effects and with random intercepts for nest identity, individual identity, day, trial and date structured in the following way. On each day we applied one of the treatments (C, RB or WP), and within days there were three types of trials (before, during, after treatment application). Trial type, treatment and their interaction were fitted as fixed effects. We fitted random intercepts for each 'day' (defined as the unique combination of experimental day and nest identity: 4 days per nest $\times$ 48 nests $-$ 13 missing days $=$ 179 unique days) to acknowledge that treatment varied at this level. We also fitted random intercepts for each 'trial' (defined as the unique combination of trial type, day and nest identity: 3 trials per day per nest $\times$ 4 days per nest $\times$ 48 nests $-$ 54 missing trials $=$ 522 unique trials) to acknowledge that type of trial (before, during, after) varied at this level. We further fitted random intercepts for 'nest identity' ($N = 48$); day (see above) was nested within nest, and trial (see above) within day within nest. We further fitted brood age (covariate) and sex (factor) as fixed effects; the random intercepts for day (above) allowed us to estimate the effect of brood age without

pseudoreplication. Similarly, we fitted random intercepts for 'individual identity' (nested within nest, $N = 96$) to acknowledge that sex varied at this level. Finally, we fitted random intercepts for 'date' ($N = 20$) to acknowledge that data from different boxes were partly taken on the same days.

The fitted interaction between treatment and trial type was expected a priori because any latency response to the model presentation over and above time-of-day effects should result in treatment-specific differences between trials. Experimental effects were only expected during trial 2 (i.e. direct effects) or trial 3 (i.e. carryover effects), but not during trial 1. We therefore applied three a priori planned contrasts, that is, we compared feeding latencies among treatment groups within each trial (for a summary of the results see also Appendix Tables A2, A3).

Temporal patterns in intervisit intervals

We analysed temporal patterns in IVIs using linear mixed-effect models with brood age, sex, trial type and treatment fitted as fixed effects and random intercepts for nest identity, individual identity, day, trial and date as detailed above for the response variable feeding latency. Here, we also added 'time' (defined below) a fixed-effect covariate. IVI was log-transformed in all models resulting in residuals not deviating from a Gaussian distribution. Time was defined as the number of seconds elapsed since the onset of the trial. Since the fixed effect 'time' varied both between and within individuals, and our interest was only in the latter effect, we applied within-subject centering (van de Pol & Wright 2009), that is, time was expressed in deviations from the individual's day- and trial-specific mean value. We also ran the model with 'right-centred' time. Time was right-centred by subtracting 30 min from all time values, so that the last IVI of a trial had the time value closest to zero and was therefore taken as the reference category for the comparison of IVI lengths. This allowed us to investigate whether IVIs at the end of experimental treatment trials 2 and 3 differed from those of the control treatment (for a discussion on centering strategies see Dingemanse & Dochtermann 2013).

An interaction effect between treatment, trial type and time was expected a priori because any changes in the length of IVI over time as a response to the model presentation should result in treatment-specific differences between trials. Treatment effects were therefore investigated by fitting a three-way interaction between treatment, trial and time. To interpret this interaction, we performed a priori planned comparisons between temporal patterns of IVIs during the C, WP and RB treatments during pretreatments (trial 1), as well as during the actual treatment (trial 2) and post-treatment trials (trial 3; for a summary of the results see also Appendix Tables A4, A5).

In the Results section we show a significant treatment*trial type interaction on how IVI decreased over time. To explore whether decreases in IVIs over time were merely caused by an effect of treatment on initial feeding latency, or whether the treatment also affected the decrease in IVI over and above the effect of the initial feeding latency, we subsequently ran the same model again, but this time with feeding latency added as a fixed effect (for individuals that came back to the nestbox within that trial, see Results). Doing so allowed us to separate temporal patterns caused by the initial response (feeding latency) from those once the birds had resumed feeding. To control for between-individual differences in feeding latencies across days, we again applied within-subject centering (see above), and expressed feeding latency in deviations from the individual's day-specific mean value. For this analysis only, we fitted a four-way interaction between treatment, trial type, time and feeding latency, as well as all lower interaction terms (see Results for rationale).

Cross-context repeatability

To test whether responses to the RB versus WP experiments reflected the same behavioural trait, we calculated the repeatability of feeding latency using linear mixed-effect models with random intercepts for individual and nestbox identity, and fixed effects of treatment (RB, WP), sex and feeding latency of the preceding trial (i.e. trial 1). Control feeding latency (during trial 1) was included as a fixed effect to account for between-individual differences in feeding latency under normal conditions. We also tested for the significance of random effects using likelihood ratio tests. In addition, we retrieved the variance components for individual and nestbox identity. Restricted maximum likelihood (REML) mixed-effects models were fitted using the rptR package, where repeatability was calculated as the between-group variance divided by the sum of the between- and within group variance (Nakagawa & Schielzeth 2010). All statistical analyses were performed in R version 2.14.2 (R Development Core Team 2012).

Ethical Note

PIT tags were injected subcutaneously between the shoulder blades. The small perforation of the skin was subsequently closed with an absorbent tissue adhesive. The procedure was done by an experienced person, did not cause any bleeding and all birds behaved normally after release. Recent studies have thoroughly investigated the use of PIT tags and found no adverse effects on adult survival and fitness in great tits and house sparrows, *Passer domesticus* (Nicolaus et al. 2008; Schroeder et al. 2011). PIT tags have been applied to our blue tits since 2007 without any evidence of long- or short-term effects on adult behaviour or body condition (Steinmeyer et al. 2010) or on nestling survival when implants were fitted 16–17 days posthatch (Schlicht et al. 2012). Permits were obtained from the government of upper Bavaria and the Bavarian regional office for forestry (LWF; no. 55.1-8642.3-7-2006; 55.2-1-54-2531.2-7-07). All experiments were carried out in accordance with the German animal protection law and were approved by the animal care and ethics representative of the Max Planck Institute for Ornithology.

RESULTS

Feeding Latency

Behavioural observations during model installation and removal indicated that both parents were typically present during experimental treatments, and that both birds engaged in extended vocal mobbing behaviour and frequent movements in the vicinity of the nestbox prior to resuming offspring provisioning. Both parents were observed engaging in this form of nest defence behaviour at 40 of 48 nestboxes (83.3%) during the WP and at 30 of 48 boxes (62.5%) during the RB presentation, whereas at least one parent was observed mobbing at 47 boxes (97.9%) during the WP presentation and at 42 boxes (87.5%) during the RB presentation.

Analyses of parental feeding latencies (detailed in Appendix Tables A2, A3) showed that neither brood age nor sex affected this response variable. There was a strong trial-specific treatment effect on feeding latency (interaction treatment*trial; Fig. 1). To investigate how the treatment*trial interaction effect came about, we examined the estimates between specific combinations of trial*treatment groups. First, feeding latencies did not differ between treatments prior to the presentation of the model (trial 1), although there was a nonsignificant trend for feeding latencies being slightly shorter during the WP than C treatment days (Table A3, Fig. 1), implying that parental feeding latencies did at least not differ strongly between experimental days under control conditions.

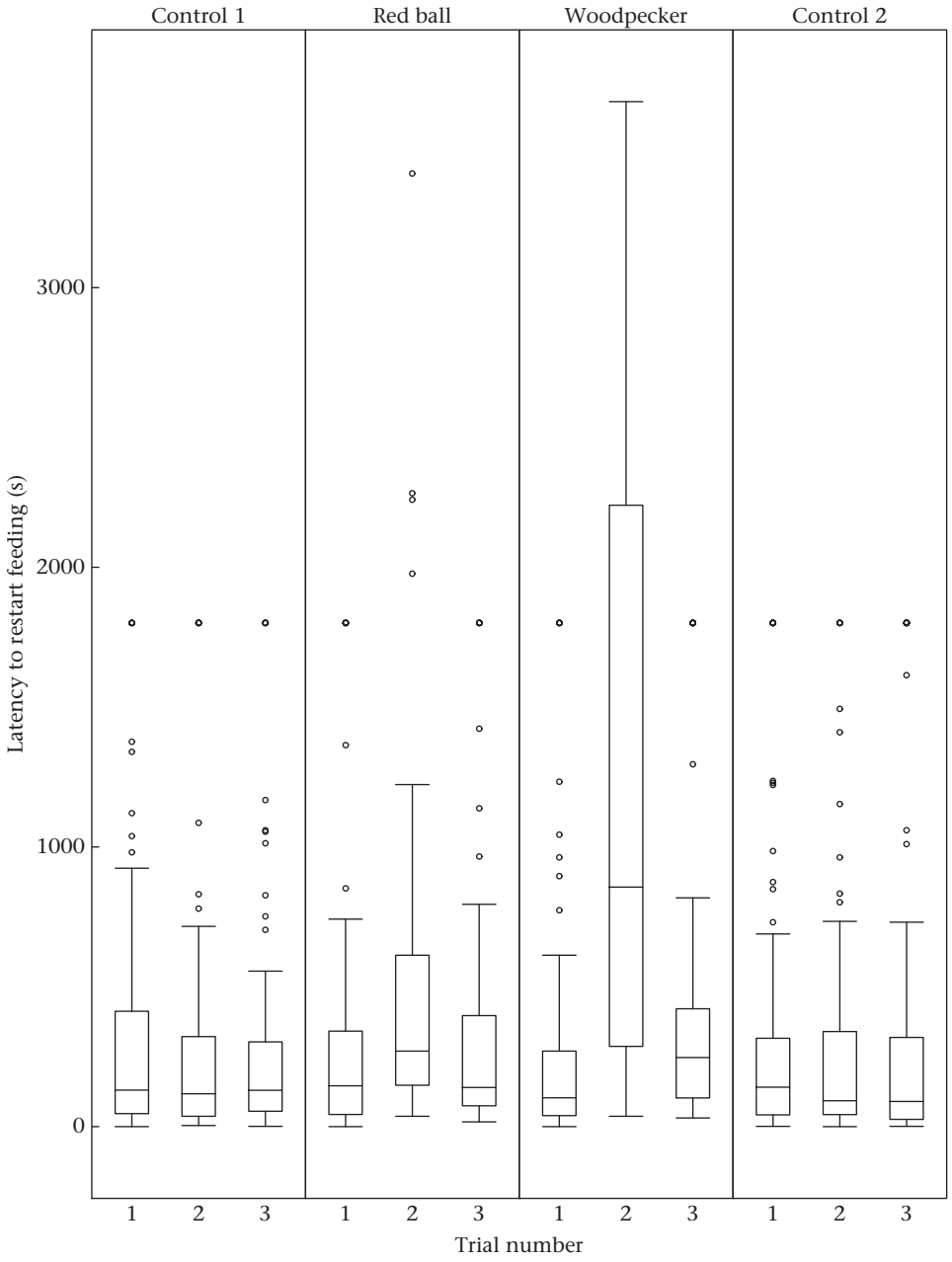

Figure 1. Latency to restart feeding during the two pre- and post-treatment controls (control 1, control 2), the red ball (RB) and woodpecker (WP) model treatments for each trial type (1–3). The RB and WP were presented at the nest during trial 2 of these treatments. Box plots show the raw untransformed values, as medians, interquartile ranges, 95% CIs and outliers.

Second, feeding latencies were significantly longer during RB and WP presentations than C treatments (trial 2; Table A3, Fig. 1), implying that the presence of the model induced parents to interrupt offspring feeding. This effect was significantly stronger for the WP than the RB treatment (Table A3, Fig. 1), implying that the birds treated the WP and RB differently. This finding came about partly because 40 of 89 individuals (45%) completely interrupted provisioning during the 30 min of the WP experimental trial, whereas only nine of 87 birds (10%) stopped feeding during the RB trial. Third, feeding latencies in the post-treatment trial (trial 3) of the RB and WP treatments were longer than those in the control post-treatments (Table A3, Fig. 1), with this effect tending to be stronger for the WP (Table A3, Fig. 1). Finally, feeding latencies during the RB and WP presentation (trial 2) were longer than those following removal (trial 3; Table A3), implying that the response to the object presentation was distinct from the effect of human disturbance.

Temporal Patterns in Intervisit Intervals

Analyses of IVIs (detailed in Appendix Tables A4, A5) revealed no differences in mean IVIs between treatments and between sexes, but IVIs decreased with increasing brood age. During the control treatment, IVIs did not change across the three trials ($F_{1,4912} = 0.19$, $P = 0.66$), implying that there was no time-of-day effect on parental feeding rates. The predicted three-way interaction between treatment, trial and time (see Methods) was indeed detected (Table A4). The a priori planned post hoc comparisons revealed that there was no difference in temporal patterns of IVIs between trial 1 of the

experimental and control treatments (Table A5, Fig. 2). Second, the presence of an object (RB or WP) in trial 2 caused the IVIs to decrease over time, whereas this was not the case during control treatments (Table A5, Fig. 2). This finding shows that parents gradually increased provisioning activity while the object was present after they had resumed provisioning. Moreover, the change in IVIs over time was more pronounced for the WP than the RB treatment (Table A5, Fig. 2). Third, IVIs during the trial following the object presentation (trial 3) also decreased more steeply over time during the WP and RB treatments than during the control treatments, and during the WP than during the RB treatment (Table A5, Fig. 2). In addition, mean IVIs during trial 3 of the WP treatment were significantly lower than those of trial 2 (Table A5). These results imply that the effect of the WP was still present after the object had been removed, with parents continuing to increase their provisioning rates, whereas this effect was much weaker for the RB treatment.

The significant treatment*trial interaction on how IVIs changed over time was confirmed in the follow-up model where we controlled for the effect of the initial feeding latency (see Methods). This showed that IVIs decreased over time with increasing feeding latency (interaction time*feeding latency: $\beta \pm \mathrm{SE} = -2.82 \times 10^{-5} \pm 9.70 \times 10^{-6}$, $F_{1,9073} = 140.49$, $P < 0.0001$), implying that the longer the individuals hesitated to restart provisioning, the steeper the subsequent increase

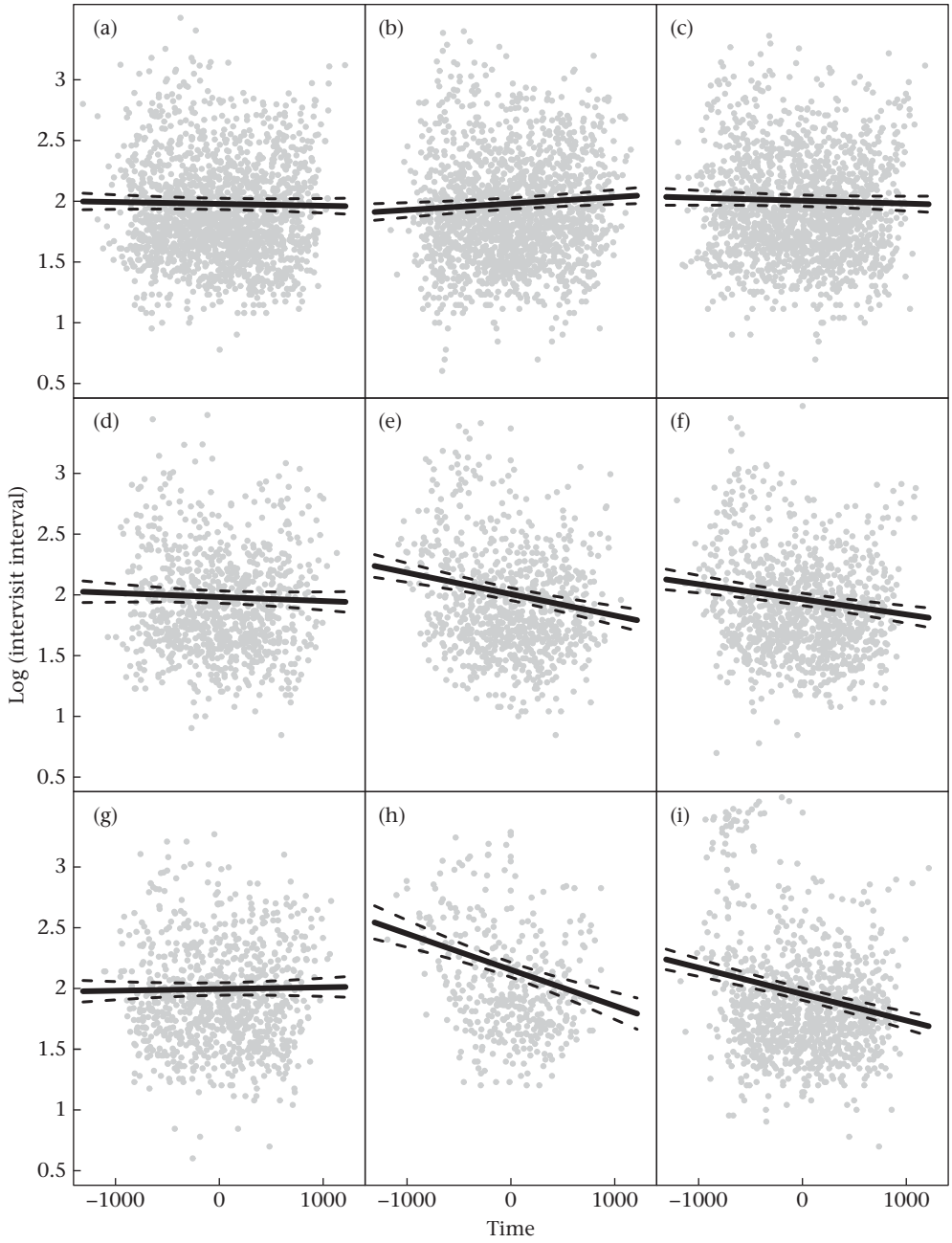

Figure 2. Parental provisioning intervisit intervals (log-transformed IVIs) against time within 30 min trials (mean-centred within individual, day and trial) for the different treatment–trial combinations: (a)–(c) control treatment trials 1–3; (d)–(f) red ball treatment trials 1–3; and (g)–(i) woodpecker treatment trials 1–3. Fitted value lines (with 95% CIs) were derived from the three-way interaction between treatment, trial and time from a linear mixed-effects model with sex and brood age included as fixed effects, and nestbox number, individual, observation and date as random intercepts (see text and Appendix Tables A4, A5). Grey dots represent the raw data. Note that IVI values can exceed 1800 s if an individual did not feed during the previous (30 min) trial; we then used the last exit of this individual at the trial before.

in their provisioning rates. This could either indicate that parents were still hesitant in approaching the nest even after their first return and/or that they were returning more often later in the trial in response to increased chick begging levels. To test which of these two explanations was supported, we investigated whether the interaction between time and feeding latency was treatment–trial specific, which was not the case (four-way interaction treatment*trial*time*feeding latency: $F_{4,9619} = 1.28$, $P = 0.28$). In other words, the effect of latency on subsequent increases in provisioning was independent of whether variation in latency was caused by the experimental treatments or not. This finding strongly suggests that these temporal decreases in IVI (Fig. 2) were driven by compensatory parental provisioning of broods that got hungry as a result of any variation in latency at the start of the trial.

Independent of these differences in feeding latencies between treatments and trials, there was a significant treatment–trial-specific effect on temporal patterns of IVIs (interaction treatment*trial*time: $F_{4,9037} = 3.48$, $P < 0.01$). This indicates that, over and above the effects of treatment that were caused by treatment–trial-specific differences in feeding latencies, IVIs during trial 2 of both experimental treatments decreased more over time than in control treatments (trial 2: RB versus C: $ß \pm SE = -1.87 \times 10^{-4} \pm 3.84 \times 10^{-5}$, $t_{9037} = -4.86$, $P < 0.0001$; WP versus C: $ß \pm SE = -2.02 \times 10^{-4} \pm 7.14 \times 10^{-5}$, $t_{9037} = -2.82$, $P < 0.01$). There was also a treatment-specific time effect for trial 3, with IVIs showing a significantly steeper decrease over time during the RB and the WP treatments than during control treatments (trial 3: RB versus C: $ß \pm SE = -9.88 \times 10^{-5} \pm 3.32 \times 10^{-5}$, $t_{9073} = -2.98$, $P < 0.01$; WP versus C: $ß \pm SE = -1.39 \times 10^{-4} \pm 3.34 \times 10^{-5}$, $t_{9073} = -4.15$, $P < 0.0001$). This implies that the relative decrease in IVIs with time (Fig. 2) was also enhanced by parents being hesitant to enter the box after the first visit of each trial above and beyond delays caused by the first latency (i.e. this hesitancy effect did not correlate with variation in latency within individuals).

The alternative model in which we used right-centering of the time variable (see Methods) revealed that IVIs at the end of trial 2 were lower in the experimental treatments than in controls (trial 2: RB versus C: $ß \pm SE = -2.07 \times 10^{-4} \pm 3.43 \times 10^{-5}$, $t_{9251} = -6.04$, $P < 0.0001$; WP versus C: $ß \pm SE = -2.96 \times 10^{-4} \pm 4.58 \times 10^{-5}$, $t_{9251} = -6.46$, $P < 0.0001$), and tended to be lower for the WP than the RB treatment (trial 2: WP versus RB: $ß \pm SE = -8.85 \times 10^{-5} \pm 5.09 \times 10^{-5}$, $t_{9251} = -1.74$, $P = 0.08$). It therefore appears as if the parents were provisioning more rapidly by the end of trial 2 in compensation for suspending provisioning at the beginning of the RB and the WP presentation, with more frequent provisioning during the WP treatment to make up for the longer latencies. At the end of trial 3, IVIs were still smaller during the experimental than during control treatments (trial 3: RB versus C: $ß \pm SE = -9.19 \times 10^{-5} \pm 3.12 \times 10^{-5}$, $t_{9251} = -2.94$, $P < 0.01$; WP versus C: $ß \pm SE = -1.72 \times 10^{-4} \pm 3.14 \times 10^{-5}$, $t_{9251} = -5.47$, $P < 0.0001$), indicating that even 30 min after the removal of the models, parents still had not completely compensated for the lost feeding opportunities induced by the presence of the WP and the RB. This effect was stronger for the WP than the RB treatment (trial 3: WP versus RB: $ß \pm SE = -7.98 \times 10^{-5} \pm 3.55 \times 10^{-5}$, $t_{9251} = -2.25$, $P = 0.02$). This was further supported by the results of a model (detailed in Appendix Tables A6, A7), comparing total number of visits during experimental and control treatments, showing that the number of visits during the WP treatment was significantly lower compared to control and RB treatments, whereas the RB treatment did not differ from the controls (Table A7).

Cross-context Repeatability

The response in terms of latency to resume feeding in the presence of a novel object and a woodpecker model was repeatable: WP and RB latencies were correlated among individuals (repeatability: $r \pm SE = 0.37 \pm 0.12$, $N = 81$, $P < 0.01$). This implies that these variables reflect, at least to some extent, individual variation in the same behaviour, which was not sex specific ($F_{1,40} = 2.54$, $P = 0.12$). In addition, a likelihood ratio test revealed differences between individuals within nestboxes (i.e. differences between the two parents) but no differences between nestboxes. This suggests that the between-individual differences in feeding latency were not caused by sex-specific roles. Individual identity explained 37% of phenotypic variation in the initial response to the novel object (feeding latency), whereas nestbox accounted for less than 1% (residual variation: 63%). At the same time, there was a significant effect of treatment ($F_{1,70} = 56.12$, $P < 0.0001$) showing that, within the same individual, feeding latencies in response to the WP were longer than those in response to the RB (WP mean ± SE: 1263 ± 115 s; RB mean ± SE: 457 ± 63 s).

DISCUSSION

This study shows that pairs of blue tits switched between alternative parental care behaviours over time according to the perceived immediate predation risk. Parents interrupted offspring provisioning in the presence of a potential threat and engaged in nest defence behaviour, but gradually resumed provisioning activity over time. The parents subsequently compensated at least partly for lost provisioning opportunities during high-risk situations by increasing their provisioning effort once the immediate threat had diminished. As part of these responses, blue tits differentiated between a potential nest predator and a novel but harmless object in an apparently adaptive manner.

Immediate Responses to Predation Risk

Provisioning blue tits showed similar behavioural responses to a potential nest predator and a novel, but nonthreatening, object. Even though we only quantified provisioning latencies, behavioural observations indicated that in both situations most blue tits immediately engaged in extended vocal mobbing behaviour and frequent movements between perches before eventually resuming offspring provisioning. Apart from effects of the model presentation, the immediate parental mobbing responses could have also been provoked by the approach of the human observer during model installation (and removal). As we do not have a proper control for human disturbance, it is difficult to tease apart these two effects in comparisons between the control and experimental treatments, although any such effects were equalized between the red ball and woodpecker treatments, thereby allowing their direct comparison. Nevertheless, blue tits typically resume normal feeding activity very quickly after a short human disturbance and often continue offspring provisioning when the observer is still within 15 m of the nest (A. Mutzel, personal observation). In contrast, during the experimental treatments the blue tits continued mobbing even after the observer had left. Mobbing may serve several purposes, namely alerting partners and offspring to the potential danger (Greig-Smith 1980; Platzen & Magrath 2004; Suzuki 2011), distracting the predator's attention away from the nest and even moving it on (Harvey & Greenwood 1978; Greig-Smith 1980), and active exploration of the predator's intention.

Even though parents initially responded to the woodpecker and the novel object in a similar way, they did delay nestling provisioning for a much longer period when confronted with the woodpecker. This finding shows that blue tits continued to consider the woodpecker model as more threatening than the red ball following the initial assessment period. As a woodpecker represents a real threat to the nestlings, such a longer delay in offspring

provisioning can be seen as adaptive (see end of previous paragraph). In contrast, mistakenly forgoing nestling provisioning for a long time in the presence of a novel, but nonthreatening, object might incur costs for the nestlings in terms of lost feeding opportunities, while nest defence behaviour against such an object is unprofitable. The initial fear of novelty, however, might still be adaptive if it allows parents to assess first whether the unknown object represents a risk, either to themselves or to their offspring. The decision of when to resume provisioning should therefore be based on the trade-off between the cost of lost nestling provisioning opportunities and the potentially high costs of wrongly judging an unknown predator as harmless.

Blue tits were clearly able to recognize certain features of the stuffed woodpecker that must be specific for this type of predator (or its taxonomic group) with their responses not merely being caused by a general fear of novel objects. Only one other study on wild birds has investigated predator recognition in an offspring provisioning context. In pied flycatchers, *Ficedula hypoleuca*, Curio (1975) showed that responses towards certain predator-specific stimuli were innate and that, as in our study, the birds also responded to unfamiliar objects, indicating that fear of novelty may also play a role in adaptive nest defence behaviour.

Our study also showed that feeding latencies in trials after the red ball removal were slightly increased compared to controls, although much less so compared to trials following the removal of the predator. This could indicate a carryover effect from the previous trial but might also have been caused by human disturbance during the removal of the object at the end of experimental trials. Human disturbance cannot, however, explain why feeding latencies were longer in the post-treatment trial of the woodpecker compared to the red ball treatment. This result implies that the predator presentation had relatively long-lasting carryover effects.

Temporal Patterns of Offspring Provisioning

Blue tit parents in our experiments resolved the conflict between the need to defend their nest against potential predators and the requirement to supply their offspring with a sufficient amount of food by adjusting different parental care behaviours over time according to the apparent level of predation threat. Even though they interrupted offspring provisioning for a certain period when confronted with a potential nest predator, 55% of the parents resumed feeding activity while the woodpecker was still present and 90% of birds did so in the presence of the red ball. The birds gradually increased their provisioning to levels above that of control treatments by the end of the trial. This gradual increase in visit rate (i.e. reduction in IVIs) represented an expected response towards greater brood demand, gradually overriding the fear of predation as time went on. Presumably, the longer the nestlings were food deprived, the hungrier they became and the louder they should have begged (Cotton et al. 1996). Future research might reveal whether such increase in offspring need with longer latencies motivated the parents to overcome fear of predation, for example by experimental manipulation of offspring begging. The strong influence of latency variation (independent of treatment) on the subsequent increase in provisioning rates at least suggests a possible role for elevated begging as the mechanism by which parents compensated for lost feeding opportunities at the beginning of all trials. This effect may simply have been magnified by the longer latencies for the woodpecker and red ball presentations.

Over and above initial latencies to resume provisioning and any subsequent compensatory increases in provisioning effort, there was also an additional gradual decrease in IVI length with time that could reflect a true habituation effect to the woodpecker (Rankin et al. 2009). This is because the longer a predator (or novel object) is sitting (stationary) in front of the nest without attacking the nestlings (or the parent), the less likely it becomes that it will attack. So, instead of one single interruption to offspring provisioning (reflected by feeding latency) followed by a quick increase in provisioning rate, parents hesitated when entering the nestbox during the first few IVIs of the experimental presentations. With increasing habituation over time, this hesitation faded, thereby creating an even steeper slope of decreasing values over time.

After the removal of the nest predator and after an initial feeding latency at the onset of the post-treatment trial, parental provisioning rates again increased to above normal (control) levels by the end of the trial. This pattern suggests that the higher brood demand caused by the experimental presentation persisted and created a carryover effect from the previous trial. It further indicates that most of the pairs that resumed provisioning during trial 2 still had not compensated sufficiently to return brood demand to pretreatment levels, and any feeding latencies at the beginning of trial 3 created still more additional brood demand to be met. Therefore, at the end of the experiment, provisioning levels were still slightly higher than controls. Had we extended the observations, this increased provisioning effort would probably eventually have returned to control levels. Such adjustments in parental effort mean that relatively short-term interruptions in provisioning (e.g. caused by the presence of predators around the nest) should have few negative long-term consequences for offspring growth and condition. However, if such disturbances occur repeatedly, these same reductions in provisioning rates over time may result in cumulative and potentially harmful consequences for offspring performance (Tilgar et al. 2011).

The temporal patterns observed during the novel object treatment did not differ qualitatively from those of the woodpecker treatment, even though most effects were not as strong for the former. The parents were apparently able to differentiate between different risk levels and adjust the strength of their behavioural response accordingly. The diminishing responses towards the disturbance by a woodpecker and a novel object further suggest that habituation might have played a role, as weaker stimuli are expected to provoke a more rapid and more pronounced habituation response (Rankin et al. 2009).

Our study clearly demonstrates that the birds' additional response towards the woodpecker model was not (merely) caused by an effect of its novelty. The comparison with a conspicuous and truly novel object such as the red ball was more informative in this regard than, for instance, the comparison with a model of another, harmless bird species. This is because only the former allows us to investigate the response to novelty and disturbance around the nest compared to the response to a potential predator.

Individual Differences in Parental Care

We detected between-individual differences in the latency to restart feeding during experimental trials. Individuals that resumed provisioning activity quicker in the presence of a nest predator model also restarted offspring provisioning earlier when faced with a novel object. This shows that the behavioural response towards a potentially threatening object close to the nest is an individual-specific trait, at least in terms of feeding latency. Such between-individual variation in how individuals cope with novel or challenging situations such as predation threats has previously been demonstrated in a wide array of taxa and is now commonly referred to as 'animal personality' (Réale et al. 2007; Dingemanse et al. 2010). Even though we did not quantify parental behaviours outside the nest, behavioural observations at the onset and the end of model presentations combined with video

recordings (from a previous year) strongly suggest that parents engaged in extended vocal mobbing towards the presented object before recommencing offspring provisioning. Nevertheless, we cannot rule out that some individuals also engaged in other activities, such as self-feeding, before restarting to feed nestlings. In addition, not only the duration but also the intensity of nest defence might have varied between individuals. Previous studies have detected consistent between-individual differences in nest defence as well as in provisioning behaviour (e.g. MacColl & Hatchwell 2003; Schwagmeyer & Mock 2003; Kontiainen et al. 2009; Redmond et al. 2009). Moreover, individual variation in parental care might also be linked to other consistent behavioural traits, such as aggressiveness or boldness (Roulin et al. 2010). For instance, aggressive males feed their offspring at a lower rate (e.g. Veiga et al. 2002; McGlothlin et al. 2007) and/or invest more in nest defence behaviour (Duckworth 2006).

Our study did not provide any evidence for a sex-specific trade-off between nest defence behaviour and offspring provisioning. This is in contrast to previous studies on avian parental care that have demonstrated positive within-pair correlations in guarding and provisioning, or a sex-specific division of tasks (male guarding, female provisioning; see Markman et al. 1995, 1996). However, the presence of between-individual differences in feeding latencies within a nest together with the finding that there was no variation among nests might still suggest the presence of a division of labour within pairs of blue tits feeding at the same nest.

Conclusions

This study revealed that blue tits adjust different aspects of their parental care behaviour over time to variation in the immediate threat of nest predation. Parents of both sexes interrupted offspring provisioning in the presence of a potential threat and engaged in nest defence behaviour. Differences in the duration of that interruption appeared linked to the possible predation threat, that is, the disturbance, novelty and unknown threat of a red ball versus the realistic ecological threat of a (model) woodpecker. In many cases, pairs gradually resumed provisioning activity, perhaps because the immobile model woodpecker and red ball failed to represent an increased or sustained threat. Parents displayed above-average feeding rates once the immediate threat had diminished. By engaging in nest defence behaviour when predation risk was high and compensating for the lost feeding opportunities during low-risk situations, blue tits appeared to show adaptive plastic shifts in parental behaviour: their behaviour may ensure immediate nestling survival and mitigate potential negative long-term consequences of feeding interruptions for offspring growth and condition. We currently lack formal theoretical treatments to predict precisely (1) the temporal trade-offs between parental behaviours described here, and (2) the circumstances under which sex-specific division of labour would be adaptive. Further studies could also investigate how changes in predation risk affect parental foraging strategies (e.g. foraging locations, rates of self-feeding, and prey types and sizes delivered during and after a nest predation threat), given that variation in predation risk might influence the trade-off between current and future reproduction.

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Appendix

To test for an effect of woodpecker model identity on feeding latency we ran a likelihood ratio test (LRT) using the restricted maximum likelihood method (REML). The LRT was constructed by comparing a linear mixed-effect model containing control feeding latency (during trial 1) as a fixed effect while also fitting random intercepts for nestbox and woodpecker model identity ('model 1') with a similar model where only random intercepts for nestbox were included ('model 2'). The LRT revealed no significant difference between model 1 and 2 ($AIC_{model\ 1} = 368.24$, $\log L_{model\ 1} = 179.12$; $AIC_{model\ 2} = 366.24$, $\log L_{model\ 2} = 179.12$; $LRT = 0$, $P = 1$). We also ran this analysis using Bayesian methods (MCMCglmm package; Hadfield 2010), confirming that the estimate was indeed close to zero (variance explained: 0.3%, 95% confidence interval, CI: 0.01–0.42%). Together, these analyses imply that there was little to no variance explained by woodpecker model identity.

To test for an effect of woodpecker identity on IVIs during trial 2, we also performed an LRT by comparing a linear mixed-effect model containing random intercepts for nestbox and woodpecker identity ('model 1') with a similar model where only random intercepts for nestbox were included ('model 2'). The LRT revealed no significant difference between model 1 and 2 ($AIC_{model\ 1} = 457.20$, $\log L_{model\ 1} = -223.60$; $AIC_{model\ 2} = 455.20$, $\log L_{model\ 2} = -223.60$; $LRT = 0$, $P = 1$). We also ran this analysis using Bayesian methods, confirming that the estimate was indeed close to zero (variance explained: 0.5%, 95% CI: 0.1–2.0%), implying that woodpecker identity did not explain any variance in IVIs.

Table A1
Effects of treatment, sex and test sequence on feeding latency

	Effect *df*	Error *df*	*F*	*P*
Intercept	1	73	2417.03	<0.0001
Treatment	1	73	59.30	<0.0001
Sex	1	40	2.44	0.13
Test sequence	1	73	0.25	0.62

Results of a linear mixed-effects model with Box–Cox-transformed feeding latency as the response variable, experimental treatment, sex and test sequence (1, 2) as fixed effects, and with random intercepts fitted for nestbox, individual, observation period number and date. Only data from trial 2 of the RB and the WP treatment were used to test whether test sequence significantly affected feeding latency during the experimental treatments.

Table A2
Analysis of variance for feeding latency

	Effect *df*	Error *df*	*F*	*P*
Fixed effects				
Intercept	1	648	2062.77	<0.0001
Treatment	2	259	39.44	<0.0001
Trial type	2	648	33.27	<0.0001
Brood age	1	259	2.37	0.13
Sex	1	47	0.34	0.56
Treatment*Trial	4	648	31.16	<0.0001
Random effects	σ^2			
Nestbox	5.38×10^{-7}			
Individual	32.09			
Day	1.43			
Date	1.43			
Trial	64.81			
Residual	0.24			

Results of a linear mixed-effects model with Box–Cox-transformed feeding latency as the response variable, experimental treatment, trial type and the interaction between treatment and trial type, brood age and sex as fixed effects, and with random intercepts fitted for nestbox, individual, day, trial and date (individual and day were nested within nestbox, and trial within day and nestbox).

Table A3
Planned comparisons among treatment groups within each trial for feeding latency

	β±SE	*df*	*t*	*P*
C treatment				
Trial 2 vs trial 1	−0.24±0.20	648	−1.18	0.24
Trial 3 vs trial 1	−0.21±0.21	648	−1.01	0.32
RB treatment				
Trial 2 vs trial 3	0.91±0.30	648	3.13	<0.01
WP treatment				
Trial 2 vs trial 3	2.22±0.30	648	7.51	<0.0001
Trial 1				
RB vs C	−0.11±0.25	259	−0.44	0.66
WP vs C	−0.44±0.25	259	−1.77	0.08
WP vs RB	−0.33±0.29	259	−1.16	0.25
Trial 2				
RB vs C	1.61±0.26	259	6.27	<0.0001
WP vs C	3.42±0.26	259	13.31	<0.0001
WP vs RB	1.81±0.30	259	6.05	<0.0001
Trial 3				
RB vs C	0.66±0.26	259	2.57	0.01
WP vs C	1.16±0.26	259	4.43	<0.0001
WP vs RB	0.50±0.30	259	1.66	0.09

C = control; RB = red ball; WP = woodpecker treatment.

Table A4
Analysis of variance for IVI

	Effect *df*	Error *df*	*F*	*P*
Fixed effects				
Treatment	2	239	0.81	0.45
Trial type	2	578	5.97	<0.01
Time	1	9251	44.25	<0.0001
Brood age	1	239	6.49	0.01
Sex	1	43	0.47	0.49
Two-way interactions:				
Treatment*Trial	4	578	12.04	<0.0001
Treatment*Time	2	9251	30.40	<0.0001
Trial*Time	2	9251	9.53	<0.001
Three-way interaction:				
Treatment*Trial*Time	4	9251	13.40	<0.0001
Random effects	σ^2			
Nestbox	6.91			
Individual	7.53			
Day	1.11			
Date	1.11			
Trial	2.99			
Residual	80.36			

Results of a linear mixed-effect model with log-transformed IVI as the response variable, with treatment, trial type, mean centred time (within individual, sequence and trial type) and brood age as fixed effects and with random intercepts for nestbox, individual, day, trial, and date (individual and day were nested within nestbox, and trial within day and nestbox). Treatment, trial type and time were also fitted as three-way and all lower term interactions.

Table A5
Planned comparisons among treatment groups within each trial for IVI

	β±SE	*df*	*t*	*P*
Brood age	$1.22\times10^{-2}\pm4.86\times10^{-3}$	239	2.51	0.01
C treatment				
Trial 2 vs trial 1	$3.44\times10^{-3}\pm1.62\times10^{-2}$	578	0.21	0.83
Trial 3 vs trial 1	$2.26\times10^{-2}\pm1.64\times10^{-2}$	578	1.37	0.17
Time				
Trial 2 vs trial 1	$6.83\times10^{-5}\pm2.72\times10^{-5}$	9251	2.52	0.01
Trial 3 vs trial 1	$-8.44\times10^{-6}\pm2.72\times10^{-5}$	9251	−0.31	0.76
RB treatment				
Trial 2 vs trial 3	$-4.70\times10^{-2}\pm2.23\times10^{-2}$	578	−2.11	0.04
WP treatment				
Trial 2 vs trial 3	$-2.06\times10^{-1}\pm2.84\times10^{-2}$	578	−7.24	<0.0001
Time				
Trial 1				
RB vs C	$-1.78\times10^{-5}\pm3.38\times10^{-5}$	9251	−0.52	0.60
WP vs C	$2.92\times10^{-5}\pm3.37\times10^{-5}$	9251	0.86	0.39
WP vs RB	$4.69\times10^{-5}\pm3.94\times10^{-5}$	9251	1.19	0.23
Trial 2				
RB vs C	$-2.29\times10^{-4}\pm3.57\times10^{-5}$	9251	−6.42	<0.0001
WP vs C	$-3.50\times10^{-4}\pm5.11\times10^{-5}$	9251	−6.84	<0.0001
WP vs RB	$-1.20\times10^{-4}\pm5.60\times10^{-5}$	9251	−2.15	0.03
Trial 3				
RB vs C	$-1.01\times10^{-4}\pm3.25\times10^{-5}$	9251	−3.10	<0.01
WP vs C	$-1.94\times10^{-4}\pm3.25\times10^{-5}$	9251	−5.96	<0.0001
WP vs RB	$-9.29\times10^{-5}\pm3.68\times10^{-5}$	9251	−2.52	0.01

C = control; RB = red ball; WP = woodpecker treatment.

Table A6
Analysis of variance for the total number of visits of each treatment

	Effect *df*	Error *df*	*F*	*P*
Fixed effect				
Treatment	2	81	15.04	<0.0001
Random effects	σ^2			
Nestbox	74.11			
Residual	25.89			

Results of a linear mixed-effect model with the total number of visits of each treatment as the response variable, with treatment as a fixed effect and random intercepts for nestbox. The total number of visits of each experimental day was calculated as the sum of visits of trial 1, 2 and 3. For the control treatment we averaged the sum of visits for experimental day 1 and 4 (i.e. control 1 and 2).

Table A7
Planned comparisons among treatment groups for the total number of visits of each treatment

	β±SE	*df*	*t*	*P*
C treatment	60.27±2.68	81	22.51	<0.0001
RB vs C	1.99±2.01	81	0.99	0.32
WP vs C	−8.62±1.99	81	−4.33	<0.0001
WP vs RB	−10.61±2.08	81	−5.11	<0.0001

C = control; RB = red ball; WP = woodpecker treatment.

Chapter 3

Parental provisioning behaviour plays a key role in linking personality with reproductive success

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Parental provisioning behaviour plays a key role in linking personality with reproductive success

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Repeatable behavioural traits ('personality') have been shown to covary with fitness, but it remains poorly understood how such behaviour–fitness relationships come about. We applied a multivariate approach to reveal the mechanistic pathways by which variation in exploratory and aggressive behaviour is translated into variation in reproductive success in a natural population of blue tits, *Cyanistes caeruleus*. Using path analysis, we demonstrate a key role for provisioning behaviour in mediating the link between personality and reproductive success (number of fledged offspring). Aggressive males fed their nestlings at lower rates than less aggressive individuals. At the same time, their low parental investment was associated with increased female effort, thereby positively affecting fledgling production. Whereas male exploratory behaviour was unrelated to provisioning behaviour and reproductive success, fast-exploring females fed their offspring at higher rates and initiated breeding earlier, thus increasing reproductive success. Our findings provide strong support for specific mechanistic pathways linking components of behavioural syndromes to reproductive success. Importantly, relationships between behavioural phenotypes and reproductive success were obscured when considering simple bivariate relationships, underlining the importance of adopting multivariate views and statistical tools as path analysis to the study of behavioural evolution.

1. Introduction

Meta-analyses have revealed that behavioural traits typically show substantial individual repeatability [1], and that individuals from the same population also vary in suites of correlated behaviours [2]. For example, individuals that are relatively active also tend to be relatively explorative and aggressive compared with less-active individuals from the same population [3]. The occurrence of such between-individual variation in single behaviours over time or across contexts is now commonly called 'animal personality' in the behavioural ecology literature [4,5], while the term 'behavioural syndrome' [6,7] refers to non-zero behavioural correlations between individuals [8]. Theoreticians have provided various explanations for why such patterns of between-individual behavioural (co)variance might result from natural selection [9–11], though few studies have yet explicitly tested their predictions and assumptions (but see [12,13]). As a consequence, we still know relatively little about why personalities and syndromes persist in nature.

In natural populations, repeatable behavioural traits similar to exploratory tendency or aggressiveness covary with proxies for fitness such as survival [14–16] or reproductive success [12,16–19], implying that behavioural phenotypes are subject to natural selection. Yet, our insight in how behavioural phenotypes affect fitness is still limited because most studies to date have estimated fitness effects of single components of syndromes, e.g. only exploratory behaviour [14,18] or only aggressiveness [20], rather than asking which components of behavioural

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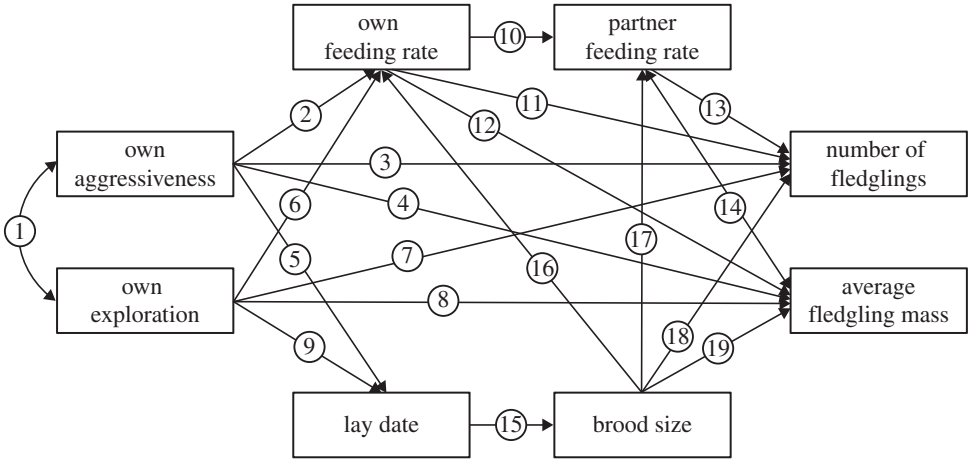

Figure 1. Hypothesized path model for males. One-headed arrows indicate the direction of hypothesized causal links. Double-headed curved arrows indicate simple hypothesized correlations. Path numbers are given in circles. We constructed the same path model for females with the exception that aggressiveness was not included because this trait was not assayed for this sex.

syndromes are directly versus indirectly under selection via their correlations with other behaviours. Moreover, we know relatively little about mechanistic pathways by which repeatable behaviour is translated into fitness, since those pathways are typically being implied rather than measured explicitly [21]. For example, is exploratory behaviour associated with reproductive success because it affects the acquisition of high-quality territories [17,22], or rather because it is associated with responsiveness towards variation in food resources [23,24] or offspring demands [25]? And which of these associations are directly due to exploratory tendency rather than representing indirect effects of aggressiveness? Such questions illustrate the necessity for integrative studies where distinct direct and indirect pathways by which components of correlated behaviours might affect fitness are simultaneously quantified [6]. This study aimed to investigate how two key behavioural traits that are often structured in a behavioural syndrome, namely aggressiveness and exploratory behaviour, affect reproductive success and how such fitness associations might come about. We therefore considered, based on previous literature, various direct and indirect pathways (detailed below), and used data collected for a passerine bird with biparental care, the blue tit, *Cyanistes caeruleus*, to assess the relative level of support for each.

We simultaneously considered the following pathways by which components of the aggressiveness–exploration syndrome would translate into reproductive success (figure 1). First, we expected that aggressiveness negatively affects feeding rate in males (path 2) [26,27], because aggressive males are generally thought to trade-off investment in offspring provisioning with investment into nest and territory defence [28,29]. Provisioning effort should also be affected by brood size (paths 16 and 17) [30], and individual parents are expected to compensate for changes in partner provisioning effort (path 10) [31]. Provisioning rates in turn should positively and directly translate into reproductive success (paths 11–14) [32]. Taken together, these relationships were expected to result in an indirect link between male aggressiveness and number (and condition) of fledglings via male and female provisioning effort.

Second, fast exploratory behaviour has been implied to positively affect the ability to acquire high-quality territories in males [17,33], and females in such territories typically lay earlier in the season than those breeding on low-quality territories [34]. We therefore expected a negative effect of male exploratory behaviour on the lay date of his social mate (path 9), whereas lay date should negatively influence brood size (path 15) [35]. Brood size, in turn, typically negatively affects fledgling weight because feeding frequency per nestling will decline with increasing brood size (path 19) [36] and was expected to positively affect the number of fledglings (path 18) [35,36]. Provided that relationships between male exploratory behaviour and acquired territory quality reported in the literature [17,22] were in fact indirect and causally mediated by aggressiveness, these relationships are expected to create a second indirect causal pathway between male aggressiveness and reproductive success via lay date (path 5) and brood size. In other words, we also expected that exploratory behaviour and aggressiveness were structured in a behavioural syndrome (path 1) [3].

For females, we do not have a formal literature-based framework of hypotheses concerning the link between exploratory behaviour and lay date. However, fast-exploring females might also be relatively aggressive and win from slow-exploring females in spring, when competing for territorial males. In line with this, fast-exploring great tit, *Parus major*, females tended to be more dominant at clumped winter food resources than slow-exploring ones [33]. Similarly, it has been shown previously that female–female aggression plays an important role in competition for breeding opportunities in blue tits [37]. We therefore hypothesized that fast-exploring females are able to acquire high-quality territories, and initiate their clutches earlier compared with slow-exploring females (resulting in a negative causal link between female exploratory tendency and lay date; path 9).

Third, we investigated how exploratory behaviour related to provisioning rates (path 6). This relationship has rarely been studied [25,38], but exploration has been directly linked to foraging abilities [24,39,40], and reproductive success in passerine birds, both in the wild [14,17–19] and in the laboratory [41]. Therefore, we predicted, for both males and females, a pathway linking exploratory behaviour with reproductive success via their own and their mate's provisioning rate (paths 6 and 10–14), with slow-exploring parents providing more parental care and therefore having a higher reproductive success than fast explorers [17].

Finally, we considered that aggressiveness and exploratory behaviour might also directly or indirectly affect reproductive success via unknown (i.e. not yet hypothesized) behavioural pathways. Thus, we tested direct (i.e. residual) pathways linking aggressiveness (or exploratory behaviour) with the proxies for fitness (paths 3, 4, 7 and 8).

2. Material and methods

(a) Study site and general field procedures

The study was carried out in a nest-box population of blue tits, in southern Germany (Westerholz, 48°08′ N, 10°53′ E) (detailed in [42]), during the breeding seasons of 2009 and 2011. From early March till the end of the breeding season, nest-boxes were checked at least once per week. We recorded lay date (date of the first egg) and hatching and fledging date, as well as the number of hatchlings (brood size) and fledglings. When nestlings were between 9 and 10 days old, both parents were caught inside the nest-box. Unbanded birds obtained a numbered metal band and a unique combination of three colour bands and were equipped with a uniquely coded passive integrated transponder (PIT) tag (EM4102 ISO animal tag 134.2 kHz ISO, 8.5 × 2.12 mm, 0.067 g), following Nicolaus *et al.* [43]. PIT tags enabled automatic monitoring of provisioning behaviour of tagged parents without direct observation of nest-boxes. At day 14, when nestlings reached their final fledging weight and size, weight and tarsus length of nestlings were measured. The number of fledglings and average fledgling weight was used as a measure of reproductive success. As parents have to trade-off investment in number versus quality (i.e. weight, [36]) of fledglings, these two measures combined represent a good proxy for reproductive success.

(b) Aggression tests

In both years of study, male aggressiveness was measured once between March and May by subjecting pairs to a simulated territorial intrusion. Final sample sizes for 2009 and 2011 were 31 and 58, respectively. A taxidermic mount of a male blue tit on a 1.5 m long wooden pole was placed 2 m from the focal nest-box. A small loudspeaker (Radio shack, Mini Audio Amplifier) connected to a MP3 player for song playback was fixed directly underneath. A snap trap was fixed on top of the pole to catch attacking males landing on the trap. Aggression tests were conducted at nest-boxes where birds had been registered frequently (either a PIT-tagged male used the box for sleeping during winter or a pair occupied the box at the onset of the breeding season). After starting song playback, the behaviour of the male territory holder was observed from a distance of 15 m until the bird was caught or for a maximum of 30 min. In total, we used six male blue tit models and five blue tit songs, randomly assigned to aggression tests to prevent pseudo-replication [44]. Neither song nor model identity affected male aggressiveness (see the electronic supplementary material, text S1). Only observations of individuals that were identified (caught during the aggression test or identified by reading colour band combinations) as the male later feeding at the focal nest (89 out of 121 tested males) were included for later analyses.

As proxy for male aggressiveness we used approach latency (for rationale, see electronic supplementary material, text S2), multiplied by −1 to obtain a continuous variable where higher values represented increased aggressiveness. We performed a box-cox transformation, resulting in models with residuals not deviating from a Gaussian distribution. Because nest stage during the aggression test significantly affected male aggressiveness (see the electronic supplementary material, table S1), this variable was controlled for in all subsequent analyses by expressing each aggression score as the deviation from the mean value for each nest stage.

(c) Exploration tests

Exploratory behaviour was measured in spring 2009 and 2011, using a cage test adapted from the classic 'novel environment test' [24,45]. Birds were captured with a snap trap during the aggression test and/or inside the nest-box when feeding nestlings (see above) and immediately brought to a car fitted with the exploration cage in the back (see the electronic supplementary material, figure S1). The behaviour of the subject was recorded for 5 min with a video camera (JVC Everio GZ-MG77E) placed 1.5 m from the cage. An individual's movements between different cage locations were later scored from videos with an event recorder (The Observer v. 5.0.31, Noldus Information Technology, The Netherlands). The total number of hops and flights within and between different locations (see the electronic supplementary material, figure S1) was used as a proxy for exploratory behaviour following the procedure outlined elsewhere [45] for the classic novel environment test in wild great tits. Repeated measures of the same individuals were used to calculate repeatability. For all other analyses, we used measures obtained during adult catching. Because nearly all parents were caught with this method, this ensured that our dataset represented an unbiased subsample of the study population.

(d) Parental provisioning behaviour

In 2011 alone, provisioning behaviour was observed at 48 nest-boxes with automatic nest-box recording devices (see the electronic supplementary material, text S3) as part of another experiment detailed elsewhere [46]. Here, we only used 'control' data of that experiment, recorded the day before and the day after the experimental treatment. For each nest, we extracted 90 min of feeding data per day on 2 days when nestlings were 11 and 14 days old. An individual's average feeding rate per hour across both observation days was used as a measure of provisioning behaviour.

(e) Statistical analyses

(i) Repeatability

Although the repeatability of exploratory behaviour and feeding rate has been demonstrated before [45,47], it has not yet been quantified for our population nor for our specific exploration test. Repeatability of both behavioural traits was calculated using univariate mixed-effect models fitted in the MCMCglmm package [48] of R v. 2.14.2 [49]. For exploratory behaviour random intercepts were fitted for individual identity, while sex and test sequence were included as fixed effects. We ran models for both years separately and for both years combined with year as additional fixed effect. To calculate repeatability of feeding rate across the two observation days, we fitted random intercepts for individual identity and sex as a fixed effect. Repeatability was calculated as the between-individual variance divided by the sum of the between-individual plus residual (i.e. within-individual) variance not accounted for by the fixed effects [50].

(ii) Path analyses

Path analysis [51] was applied to infer how proxies for short-term fitness were directly versus indirectly related to behavioural traits (i.e. aggression and exploration), parental investment (provisioning rate) and female reproductive decisions (lay date and clutch size). Since provisioning data were not available for 2009, only data from 2011 were included. We estimated the variance–covariance matrix between all hypothesized predictor and response variables and took the estimated matrices forward for path analyses. We ensured that the uncertainty around the

estimates was appropriately taken forward by applying a Bayesian framework with Markov chain Monte Carlo (MCMC) methods. Variances and covariances were derived by fitting two multivariate models (MCMCglmm package; see electronic supplementary material, text S4 for details on prior specifications), one for each sex, with aggression (males only), exploratory behaviour, lay date, brood size, the focal individual's and partner's feeding rate, and fledging number and weight as response variables (see the electronic supplementary material, tables S2 and S3). Excluding aggressiveness from the male model, to make it directly comparable to the female model, did not result in any changes in the model outcome (results not shown).

The key advantage of the MCMC method is that the model output gives the entire posterior distribution of each fixed and random parameter. This distribution can subsequently be used for further analyses, such that the uncertainty around point estimates is appropriately taken forward. Path analyses were performed within the structural equation modelling package in R [52], where we applied a Bayesian framework by running each of the specified path models once for each of the 1000 estimated variance–covariance matrices (see above). Using these 1000 runs, we calculated for every specified path the most likely path coefficient value and its associated 95% credible interval. Credible intervals not including zero indicate statistical significance. For intervals only slightly overlapping zero, we calculated how often the estimate was positive or negative, thus giving a value that is comparable with a *p*-value [53]. Path coefficients for compound paths were estimated to infer the level of statistical support for indirect effects which was achieved by multiplying all coefficients along the focal path [51]. Path analysis allows for the calculation of partial correlation coefficients between two variables while simultaneously controlling for effects of all other variables in the model [51]. This makes it a powerful tool to disentangle direct from indirect effects (i.e. produced by the effect on another correlated variable). This is of particular importance for datasets where predictor variables are assumed to be highly inter-correlated [51], as in behavioural syndrome studies (see also [54]). As paths between the variables were hypothesized *a priori*, we present the results for the full model, which also includes the paths not supported by the model [55,56].

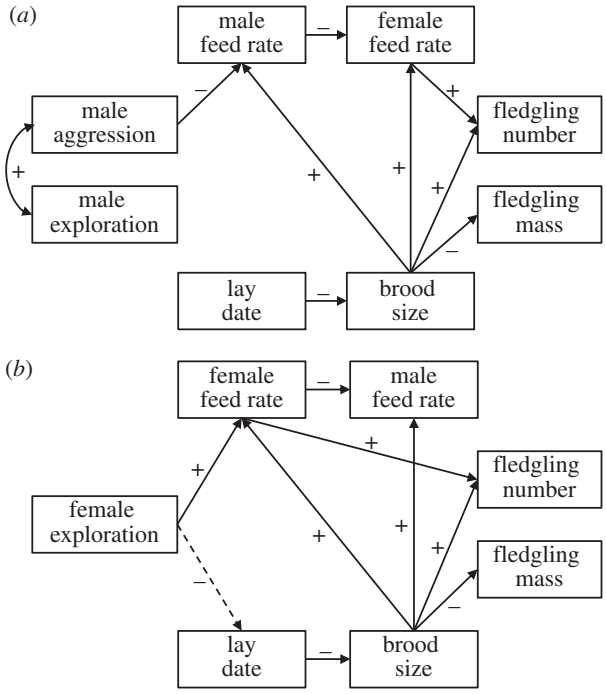

Figure 2. Supported paths in models for (*a*) male and (*b*) female blue tits. Only paths with considerable statistical support are shown. Black arrows indicate strong support (credible intervals not overlapping zero), dashed black lines indicate some support (credible intervals slightly overlapping zero but with $p < 0.05$).

3. Results

(a) Repeatability of behaviour

Exploratory behaviour was repeatable in 2011 ($R = 0.60$, 95% CI: 0.13, 0.79), and also when data of both years were combined ($R = 0.66$, 95% CI: 0.44, 0.76; the statistical model did not converge for 2009; electronic supplementary material, table S4). Feeding rate was significantly repeatable across the two observation days ($R = 0.78$, 95% CI: 0.64, 0.83; electronic supplementary material, table S4). These findings imply that individuals consistently differed in how they explored a novel environment and how often they fed their offspring.

(b) Parental feeding rates, breeding decisions and reproductive success

The variance–covariance matrices calculated separately for males and females were largely based on the same parental feeding rates, lay date, brood size and reproductive success data. Therefore, path models for both sexes revealed similar patterns for all pathways including these variables. There was strong support for a negative pathway linking an individual's and its partner's feeding rate (figure 2*a*,*b*; table 1, path 10), suggesting that low investment in offspring provisioning of one pair member was associated with a higher partner effort. Even though male feeding rate did not directly affect fledgling production (table 1, path 11 for male model, path 13 for female model), it indirectly and negatively affected fledging success via female parental effort (figure 2*a*,*b* and table 1, compound path A), implying that female provisioning behaviour was more important for nestling survival. The path model further provided strong support for a trade-off between offspring quality and quantity, mediated by brood size, which positively affected fledgling production both directly (figure 2*a*,*b* and table 1, path 18), and indirectly (via female feeding rate; figure 2*a*,*b* and table 1, compound path B). At the same time, brood size directly and negatively influenced average fledgling mass (figure 2*a*,*b* and table 1, path 19). Furthermore, the path model supported a significant negative pathway linking lay date with brood size (figure 2*a*,*b* and table 1, path 15), providing strong support for our hypothesis for a direct link between these variables: females initiating egg laying earlier also had larger broods.

(c) Male behaviour and reproductive success

The path model for males supported a link between aggressiveness and exploratory behaviour, with more aggressive individuals exploring a novel environment faster than less aggressive ones (figure 2*a* and table 1, path 1). At the same time, there was a negative link between aggressiveness and male feeding rate, with aggressive males feeding their offspring at a relatively low rate (figure 2*a*; table 1, path 2). The model further revealed an indirect positive effect of male aggressiveness on fledgling production via male and female feeding rates (table 1, compound path C).

Table 1. Estimated partial regression coefficients for male and female path models. The estimate of a path coefficient of a compound path (containing more than one path) is the product of the coefficients along its path. Bold numbers indicate path coefficients (path coef.) that are strongly supported by the model (95% credible interval CI not overlapping zero). Italic numbers indicate path coefficients that have some support from the model (credible intervals slightly overlapping zero but with $p < 0.05$).

		males			females		
path number	hypothesized link	*n*	path coef.	95% CI	*n*	path coef.	95% CI
1	aggression → exploration	41	**0.27**	0.01, 0.54	—	—	—
2	aggression → own feed rate	33	**−0.49**	−0.64, −0.09	—	—	—
3	aggression → fledgling no.	45	−0.06	−0.25, 0.17	—	—	—
4	aggression → fledgling mass	44	−0.27	−0.52, 0.03	—	—	—
5	aggression → lay date	43	0.23	−0.12, 0.52	—	—	—
6	exploration → own feed rate	44	0.22	−0.17, 0.38	37	**0.55**	0.12, 0.68
7	exploration → fledgling no.	60	0.10	−0.11, 0.23	56	−0.12	−0.29, 0.08
8	exploration → fledgling mass	59	0.03	−0.21, 0.24	55	−0.07	−0.32, 0.22
9	exploration → lay date	58	−0.20	−0.54, 0.12	54	*−0.38*	−0.62, 0.02
10	own feed rate → partner feed rate	48	**−0.59**	−0.83, −0.32	48	**−0.56**	−0.76, −0.35
11	own feed rate → fledgling no.	48	0.19	−0.14, 0.45	48	**0.52**	0.24, 0.74
12	own feed rate → fledgling mass	48	−0.14	−0.47, 0.32	48	0.23	−0.17, 0.59
13	partner feed rate → fledgling no.	48	**0.43**	0.22, 0.69	48	0.17	−0.10, 0.44
14	partner feed rate → fledgling mass	48	0.16	−0.20, 0.46	48	0.03	−0.42, 0.35
15	lay date → brood size	67	**−0.52**	−0.64, −0.25	71	**−0.50**	−0.69, −0.34
16	brood size → own feed rate	48	**0.41**	0.17, 0.61	48	**0.66**	0.46, 0.84
17	brood size → partner feed rate	48	**0.56**	0.38, 0.80	48	**0.29**	0.03, 0.50
18	brood size → fledgling no.	69	**0.52**	0.29, 0.72	73	**0.53**	0.32, 0.75
19	brood size → fledgling mass	66	**−0.53**	−0.85, −0.27	69	**−0.47**	−0.82, −0.18
compound path	individual path numbers						
A	10 × 13		**−0.28**	−0.46, −0.09		—	—
B	17 × 13 (males); 16 × 11 (females)		**0.28**	0.14, 0.44		**0.10**	0.02, 0.26
C	2 × 10 × 13		**0.09**	0.01, 0.23		—	—
D	1 × 2		*−0.06*	−0.26, 0.02		—	—
E	6 × 11		—	—		**0.15**	0.02, 0.39
F	9 × 15		—	—		**0.10**	0.02, 0.26
G	9 × 15 × 18		—	—		*0.09*	−0.01, 0.19
H	9 × 15 × 19		—	—		*−0.06*	−0.21, 0.02

The link between male aggressiveness and female lay date was not supported by the path model (table 1, path 5), thereby failing to confirm the hypothesis that aggressive males derive fitness benefits from having higher quality territories (or mates). There was no evidence for any unknown pathway, since the direct (i.e. 'residual') pathways between aggressiveness and reproductive success (table 1, paths 3 and 4) were not (strongly) supported.

To assess whether there was an overall effect of male aggressiveness on reproductive success, we investigated the raw phenotypic correlations derived from the multivariate model specified earlier. We found no support for an overall effect of male aggressiveness on fledgling production ($r = 0.06$, 95% CI: −0.28, 0.27; electronic supplementary material, table S2). At first glance, this result might seem surprising, as the correlation should reflect the sum of all path coefficients and the path model only supported a positive indirect link between aggression and fledgling production mediated via parental feeding rates. There are two explanations. First, the correlation between aggressiveness and fledgling production was not zero, but was not detected due to lack of power (type II error). Yet, the estimate was close to zero and its credible interval was largely overlapping zero, making this explanation unlikely. Second, other counteracting pathways

might have been present but were not strongly supported because their effects were subtle, inherently resulting in a lack of power given the data at hand. This explanation is more likely, since all other paths indeed had negative point estimates, suggesting that aggression might have negatively affected fledgling production via female lay date, male feeding rate and an unknown (residual) pathway, jointly cancelling out the positive effect of indirect pathways via the birds' provisioning rates.

Finally, between male variation in exploratory behaviour did not directly affect male provisioning rate (table 1, path 6) or female lay date (table 1, path 9). The negative indirect link between exploratory and provisioning behaviour, mediated by the covariance between exploratory behaviour and aggression was weak, but significant ($p = 0.04$; table 1, compound path D). There was little support for an unknown pathway, since direct (i.e. 'residual') pathways between exploratory behaviour and reproductive success were not supported (table 1, paths 7 and 8). In other words, in this population, links between exploratory behaviour and reproductive parameters are likely solely caused by its correlation with aggressiveness, implying that exploratory behaviour was an indirect rather than a direct target of selection.

(d) Female behaviour and reproductive success

In females, there was support for two distinct indirect effects of exploratory behaviour on reproductive success. First, fast-exploring females fed their nestlings at a relatively high rate (figure 2*b* and table 1, path 6), resulting in a positive indirect effect of female exploratory tendency on fledgling production (table 1, compound path E). Second, female exploratory behaviour affected the timing of reproduction, with fast explorers initiating clutches relatively early in season having larger broods (table 1, compound path F), resulting in a slight positive effect on fledgling production and a slight negative effect on fledgling mass ($p = 0.04$, table 1, compound paths G and H). Even though there was no strong support for an overall effect of female exploratory behaviour on fledgling production, the positive correlation coefficient between these variables ($r = 0.18$, 95% CI: -0.13, 0.35; electronic supplementary material, table S3) suggests that such a link remained undetected due to a lack of power (see above).

4. Discussion

We showed that aggressive males fed their nestlings at a lower rate than less aggressive ones. Their low feeding rate was associated with an increased female effort resulting in a positive effect on fledgling production. Our study also revealed a sex-specific link between exploratory behaviour and reproductive success. Fast-exploring females, but not males, had a higher fledging success than slow-exploring ones, mediated by both a higher feeding effort and an earlier lay date.

(a) Aggressiveness and reproductive success

As expected, aggressive males fed their offspring less often than non-aggressive ones. There was nevertheless a positive effect of male aggressiveness on fledgling production mediated by parental feeding rates. This was because low male provisioning effort was directly associated with an increased female effort, which in turn positively affected fledgling production. There are several explanations for this finding. First, the behavioural trade-off between different aspects of parental care might be resolved by a division of labour between members of a pair. For instance, aggressive males might invest more time and energy in territory or nest defence [26,57], thereby increasing offspring survival [58], whereas their female partners mainly focus on offspring provisioning. To test this hypothesis, it would be necessary to not only measure feeding rate but also other aspects of parental care, such as nest defence behaviour. Second, as parental care is costly [32], both members of a pair benefit from investing less than the partner, creating a conflict between parents over care [31,59]. This conflict is assumed to lead to a negotiation between partners where an individual benefits from adjusting its care directly in response to its partner [60]. Aggressive males might be better in 'winning' this conflict, reducing their own investment in parental care at the expense of their female partner. However, the outcome of this conflict is predicted to be evolutionarily stable only if the partner partially compensates for the shortfall of the other pair member [31,60,61]. In our study, females instead overcompensated for low feeding rates of aggressive partners, implying that this hypothesis on its own cannot explain these findings. Third, aggressive males could provide females with other benefits apart from help with offspring provisioning. For instance, aggressive males might be of higher genetic quality, passing on the good genes to their offspring. As such high-quality offspring are more valuable, females paired with these males might be willing to increase investment in current at the expense of future reproduction [62,63]. Aggressive males might also be better in acquiring and defending high-quality breeding territories [17], facilitating foraging for females and thus permitting males to reduce their own investment in offspring provisioning. However, male aggression was not associated with female lay date, suggesting that male territory did not play an important role. Overall, our study indicates that division of labour as well as male quality might be involved in mediating the link between aggression and reproductive success. Yet, experiments are now needed to test the predictions of these hypotheses.

(b) Exploratory behaviour and reproductive success

Male exploratory behaviour and feeding rate were not associated, thereby contradicting repeated suggestions that exploratory behaviour affects parental care [17,25]. Possibly, male exploratory behaviour, rather than impacting feeding rates, affects other aspects of provisioning behaviour that were not included in our study, such as prey type or load size [64]. For example, there is individual variation in both visit rate and load size in blue tits [65]. The finding that male—in contrast to female—feeding rate did not affect fledgling production must imply that male feeding rate alone was not a good predictor of the amount of food brought to the nest. For instance, fast-exploring males might cover larger distances while foraging [23], thus reaching less depleted food patches with more profitable (e.g. larger) prey items [66]. Alternatively, they might be more selective in prey choice, thus spending more time searching for food. Both options would lead to fast explorers visiting the nest less often, but delivering larger prey items. However, if larger prey sizes would

completely compensate for lower feeding rates, one would not expect the demonstrated negative link between male and female feeding rates. It thus remains to be tested whether variation in prey size plays an important role in determining provisioning efforts in male blue tits.

Female exploratory behaviour was a good predictor of feeding rate. Fast-exploring females fed their offspring at a higher, not at the expected lower rate, than slow-exploring ones. At the same time, female feeding rate positively affected fledgling production, indicating that, at least in females, feeding rate was a good predictor for the total amount of food brought to the nest and that other aspects of provisioning behaviour played a less important role compared with males. Earlier work showed that slow explorers were more flexible and better at locating new food sources, and therefore adapted more easily to changing and harsh environmental conditions [24,39]. Our study population breeds in a high-quality habitat (mature oak forest) and nesting success in the year of the study was relatively high, indicating that the caterpillar peak matched well with nestling feeding peak. Under such favourable environmental conditions, fast explorers might forage more efficiently than slow-exploring birds [14,67], allowing them to provision their offspring at a higher rate. The finding that fast-exploring females invested more in parental care also provides some support for current theoretical life-history trade-off models predicting that aggressive, fast-exploring and risk-taking individuals have moderate future fitness expectations and therefore invest more in current reproduction, whereas slow-exploring and relatively risk-averse individuals should have better future expectations and therefore invest less in current reproduction [68,69]. Possibly, this effect was not found in males, because other aspects of parental care, besides feeding rates, better indicate male investment in current reproduction. For instance, aggressive males might defend their territory or nest more vigorously than non-aggressive ones, thereby permitting females to concentrate on offspring provisioning.

(c) Assortative mating and behavioural syndromes

Aggressive males, which were also fast explorers, had mates that fed their offspring at a high rate. At the same time, fast-exploring females generally fed their offspring more often. This could result in sexual selection favouring assortative mating with respect to exploratory behaviour, as has previously been suggested for great tits and other species [70]. In addition, it has been shown previously that assortatively mated pairs produced fledglings in the best condition [17,41] had a higher fledging success [71] and recruited more offspring than non-assortative pairs [14]. However, fitting male aggressiveness and male and female exploratory behaviour into a single path model did not reveal any covariance between male and female behaviour (results not shown), implying that the detected effects were not caused by assortative mating, but were rather caused by an individual's own behavioural phenotype.

Our study further revealed the expected positive relationship between male aggressiveness and exploratory behaviour. Such an aggression–exploration syndrome has previously been shown for a variety of taxa [7] and has been suggested to emerge when individuals of the same population use different behavioural strategies to cope with stressful situations [72]. This particular behavioural syndrome has been predicted to include behaviours related to parental care [25]. Interestingly, we showed that only aggressiveness, but not exploratory behaviour, was directly linked to nestling provisioning. This finding implies that despite their covariance, the two behavioural traits were nevertheless sufficiently distinct to allow detection of distinct behaviour-specific effects on reproductive success. Our results thus suggest the existence of at least two independent proximate mechanisms involved in driving the variation in these two behavioural traits. An alternative explanation would be that our measurement error is so substantial that any correlation between the two behaviours would be underestimated.

The present study focused on phenotypic correlations between individuals documented within a single season, implying that we cannot currently ascertain that the reported paths represent long-term between- as opposed to within-individual relationships [73]. Between-individual correlations result from differences in average behaviours between individuals caused by variation in genetic constitution and permanent environment effects, whereas within-individual correlations result from correlated plastic behavioural responses to environmental conditions [73]. As part of another study in great tits, we are therefore currently collecting data on the same individuals for multiple years, which allows us to partition pathways similar to those reported in the current study into between- and within-individual components [73]. Another consequence of our focus on a single year is that we were not able to evaluate whether the reported relationships (selection pressures) were general versus year-specific. Indeed, year-to-year variation in selection pressures acting on behavioural traits, such as aggressiveness, sociability and exploratory behaviour have been documented in a range of taxa [21]. Addressing the stability of the variance–covariance matrix and its associated underlying pathways will thus represent an exciting avenue for future research, and might shed light on the outstanding question of how animal personality variation is maintained in natural populations.

5. Conclusions

This paper revealed the mechanistic pathways by which aggressiveness and exploratory behaviour were affecting reproductive success. The reported mechanisms would have remained undetected if we had failed to apply a multivariate perspective on behavioural evolution [54,74], thereby illustrating the added value of holistic approaches towards the study of adaptive evolution [6].

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Chapter 4

Experimental manipulation of parental work load reveals personaliy-related differences in the willingness and ability to raise offspring

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ABSTRACT

Animals from a wide range of taxa show repeatable individual differences in behaviour. Moreover, these behavioural traits often covary with proxies for fitness, such as survival and reproductive success, implying that these traits are subject to natural selection. Yet, we still know very little about how behavioural differences between individuals (cf. personality) are translated into variation in fitness, i.e. what mechanistic pathways are involved in linking personality with fitness. Moreover, our understanding of the relationship between personality and reproductive success is often hampered by the observational nature of most studies. This is because links between personality and reproductive success might be obscured, when behaviour phenotypes differ in other aspects that also affect reproductive success, e.g. habitat quality or brood size. To break apart such potential personality-environment correlations, we experimentally manipulated brood sizes into three brood size categories (reduced, control, enlarged). Using a phenotypic selection approach, we quantified the selection gradients acting on exploratory behaviour in natural populations of great tits, *Parus major*. We found evidence that exploratory behaviour covaried with reproductive success. Yet, selection on behavioural phenotypes differed between brood size manipulation groups with fast explorers being better in raising a high number of chicks than slow-exploring parents. Moreover, selection pressures also differed across years and sexes. This suggests that heterogeneous selection might be an important mechanism for maintaining behavioural variation. By applying path analysis, we integrated the role of different aspects of parental provisioning behaviour in translating variation in exploratory behaviour into fitness. We show that parental feeding rate did not play a role in mediating links between behaviour and reproductive success. Possibly, this was because behavioural phenotypes differed in other aspects, e.g. the type of prey delivered to nestlings or habitat quality, which are more important for determining reproductive success in our great tit populations. This work emphasizes the importance of experimental

studies when investigating personality-fitness relationships. Further experimental studies are now needed to investigate the role of parental care and other related and unrelated mechanisms in mediating links between behaviour and fitness.

INTRODUCTION

Animals continuously adapt their behaviour to changes in their environment in order to maximize their fitness. At the same time, individuals also typically show repeatable differences in their behaviour (Bell et al. 2009), since each individual does not express the full range of behavioural trait values of its population (Réale & Dingemanse 2010). Between-individual variation in behaviour, commonly referred to as 'animal personality' in the behavioural ecology literature (Dingemanse et al. 2010; Réale et al. 2010), has been shown to be heritable (Stirling et al. 2002; Dingemanse & Dochtermann 2013; van Oers & Sinn 2013), implying that it may evolve in response to selection. One of the key questions in personality research is whether personality structure imposes constraints on adaptive evolution (Dochtermann & Dingemanse 2013) or whether personality structure might itself be favoured by natural or sexual selection (Dall et al. 2004; Wolf & Weissing 2010).

Whereas there is accumulating evidence that behavioural traits covary with fitness components, such as survival or reproductive success (Dingemanse & Réale 2005; Réale et al. 2007; Smith & Blumstein 2008; Dingemanse & Réale 2013), we know relatively little about mechanistic pathways by which behavioural differences between individuals (cf. personality) are translated into variation in fitness. Suggested mechanisms involve personality-dependent differences in foraging ability (Verbeek et al. 1994; Marchetti & Drent 2000; van Overveld & Matthysen 2010), resource defending potential (translating into territory quality) (Verbeek et al. 1996; Dingemanse & De Goede 2004; Scales et al. 2013) and responsiveness to external cues (e.g. towards variation in offspring demand; Roulin et al. 2010). For

instance, there is evidence that fast-exploring great tits are able to acquire high-quality habitats (Dingemanse & De Goede 2004; Both et al. 2005). This could result in fast-exploring females laying larger clutches as they have more resources available for egg production (Boyce & Perrins 1987), and/or in fast-exploring parents provisioning their nestlings at a higher rate as foraging should be easier when food availability is high. On the other hand, slow-exploring individuals have been shown to be more sensitive towards changes in their environment, such as variation in food resources (Verbeek et al. 1994; Drent & Marchetti 1999; van Overveld & Matthysen 2010). In the breeding season, great tits mainly forage in unstable food patches distributed across their territories (e.g. Naef-Daenzer & Keller 1999), and prey abundance and size varies both within trees and within days, forcing parents to continuously sample the trees within their territory (Grieco 2001). Their flexibility should enable slow explorers to forage more efficiently compared to fast-exploring individuals, especially under harsh or unstable environmental conditions when food availability is already low. All of these pathways may thus affect fledgling number and condition. Yet, the relative importance (strength) and direction of these pathways, and their dependence on environmental conditions remains largely unstudied (Mutzel et al. 2013).

One variable that is likely to play an important role in mediating links between personality and reproductive success is parental care. This is because parental care directly affects reproductive output (Clutton-Brock 1991), while levels of parental care are expected to differ between behavioural phenotypes (Wolf et al. 2007; Roulin et al. 2010). Yet, potential links between personality, parental care and reproductive success might be obscured by behavioural phenotypes differing in other aspects that simultaneously affect brood size and parental care. For instance, habitat quality affects the resources available for reproduction (i.e. egg production), and subsequent brood sizes consequently affect parental investment. In other words, the observed brood sizes may not be independent of an individual's behavioural phenotype and/

or the habitat, where an individual breeds. Slow explorers, for example may choose to breed in low-quality territories, where competition is relatively weak, but their good foraging abilities should allow them to nevertheless lay as many eggs and to raise an equal amount of chicks as fast explorers.

In addition, there might be physiological constraints on egg production (Monaghan & Nager 1997; Visser & Lessells 2001), obscuring any link between personality and parental effort. For instance, by investing more time and energy in the current brood, fast explorers might be able to raise more nestlings than slow-exploring individuals (Wolf et al. 2007; Biro & Stamps 2008), but this link might remain undetected if there are physiological constraints hindering fast-exploring females to lay their optimal clutch size (Monaghan & Nager 1997). To break apart such potential personality-environment correlations, it is necessary to manipulate brood sizes in order to confront different behavioural types with the entire range of brood size variation. Such experimentally induced variation in brood demand consequently enables us to test which parenting types do well versus poorly under which type of condition. Moreover, if brood sizes are not manipulated, it might be hard to reveal a relationship between personality and provisioning behaviour. This is because natural brood sizes usually follow a normal distribution with few cases at the extreme ends. Increasing the total variation and flattening the distribution of brood size by manipulation, allows estimating the effects of relative fitness over a broader range of brood size values (Schluter 1988).

We used a natural population of great tits to investigate in detail how exploratory behaviour is related to reproductive success, what behavioural mechanisms are involved in mediating these links and whether the selection on parenting and exploratory behaviour is heterogeneous. We experimentally determined the brood size that each individual was given to raise (reduced, control or enlarged). The experimentally manipulated brood demand thus enabled us to test which behavioural phenotypes do well versus poorly under which type of condition. Moreover, the

ability or willingness to successfully raise nestlings should also be a function of the type of year. While food availability was extremely low in the first year of the study, resulting in extremely low reproductive success ('bad' year), food was abundant in the second year ('good' year), resulting in high nestling survival.

We considered the following pathways by which exploratory behaviour would translate into reproductive success. First, we predicted that exploratory behaviour affects different aspects of provisioning behaviour, thereby indirectly affecting reproductive success. As fast-exploring individuals are expected to invest more in the current brood, they should be better able to cope with enlarged brood sizes. Moreover, as provisioning of nestlings is more costly when environmental conditions are poor, we expected these links to be strongest in the 'bad' year. Second, we hypothesized that fast explorers should lay larger clutches mediated by an earlier lay date. This is because fast explorers are expected to be able to acquire high-quality territories, allowing them to initiate breeding relatively early in the season. Overall, we predict that fast-exploring parents should be the better parents, with this effect becoming most apparent under challenging conditions (poor food availability and high brood demand).

MATERIAL AND METHODS

Study site and general field procedures

The study was carried out in 12 nestbox populations of great tits, located south of Munich, Germany (47º55' - 48º01'N, 11º09' - 11º20'E), during the breeding seasons of 2010 and 2011. Each of the 12 plots consisted of 50 nestboxes arranged on a 50 × 50 m grid. Taken together the plots cover a total area of about 120 ha of mixed woods with beech, *Fagus sylvatica*, as the predominant tree but also containing spruce, *Picea abies*, and smaller patches of maple, *Acer platanoides*, ash tree, *Alnus sp*., larch, *Larix decidua*, and other deciduous tree species. From early April till the end

of the breeding season (July/August), we checked nestboxes at least twice per week to record lay date (date of first egg), onset of incubation, hatching and fledging date as well as hatchling and fledgling number for each brood. When the nestlings were 3 days old, we manipulated brood sizes of nests such that individuals had to raise either a reduced (-3 nestlings), a control (swapping of 3 nestlings, but no change in brood size), or an enlarged brood (+3 nestlings). Nestlings were exchanged between nests of similar age and brood mass (i.e. of similar condition) and which were within 10 min travelling distance. Nests, for which there was no matching brood remained unmanipulated. When the nestlings were between 7 and 11 days old, we caught both parents inside the nestbox with a spring trap. Individuals that were not previously banded were fitted with a numbered metal band and a unique combination of three colour bands. Nestlings were weight and measured (tarsus and wing length), at an age of 14 days, after having reached their final fledging weight and size. We used the number of fledglings and the average fledgling weight of a brood as a measure of reproductive success (Mutzel et al. 2013).

Behavioural assays

Exploratory behaviour

We measured exploratory behaviour of 166 individual great tits (77 in 2010, 89 in 2011, 80 females, 86 males) captured inside nestboxes during the general field procedures. The exploration test was conducted inside a cage and can be considered as a version of the classic 'novel environment test' (Verbeek et al. 1994; Dingemanse et al. 2002). The procedure was as follows: Directly after capture, birds were transferred to a small darkened plastic box connected to the exploration cage via a sliding door to recover from handling stress. After one minute, the bird was released into the cage without handling by opening the sliding door towards the experimental cage and moving a piece of cloth in front of the transparent opposite side of the small box. We recorded the behaviour of the subject for two minutes with a video

camera (JVC Everio GZ-MG77E) that was placed at a distance of two meters in front of the cage. The exploration cage consisted of a solid plastic box (61L × 39W × 40H cm) with one mesh side and was equipped with three perches (add picture in Suppl. Material). The individual's movements between perches, front mesh wall (6 sections) and floor (three sections) were later scored from videos recordings. The total number of flights and hops within and between different locations were used as a proxy of exploratory behaviour following procedure outlined by Dingemanse et al. (2002) for the classic novel environment test. In blue tits, exploratory behaviour measured inside a cage, has already been shown to be correlated with aggression (Mutzel et al. 2013), boldness (Kluen et al. 2012), parental feeding rate (Mutzel et al. 2013), and exploratory tendency in the wild (Herborn et al. 2010). In our great tit population, level of activity inside the cage during the breeding season predicts boldness in winter (Stuber et al. 2013), implying that it reflects exploratory behaviour rather than general activity (see also Dingemanse et al. 2002). Each individual was tested once per brood. The average between-observer correlation coefficient of the video analysis, calculated from 10 videos analysed by each observer (n=8), was 0.93 (range: 0.75 – 0.99).

Parental provisioning behaviour and chick begging levels

Provisioning behaviour of both parents and begging levels of individual nestlings were recorded at 87 nestboxes (41 nestboxes in 2010 and 46 in 2011) between 8h00 and 10h00 when the nestlings were 12 days old. Two days before the actual recording took place, we installed a small infrared camera (CDD Bird Box Camera with IR Night Vision 420TV lines) by exchanging the side door of the nestbox with a small wooden box containing the camera. At the same time we raised each nest by 2 cm by inserting a piece of foam underneath the nest material, to ensure that the camera captured the entire nest cup. To prevent parents to sit inside the side box, we inserted a plexiglass between the nest and the box. On the following

day, nestlings were weighed and marked uniquely by applying different symbols on the upper part of the head of each nestling with water soluble acrylic paint (as part of another experiment). On the actual recording day, we connected the nestbox camera to a portable recording device (Archos 5 Internet Media Tablet) and a power supply placed at a distance of 20 m from the nestbox. After switching the camera on, we left the immediate nest environment (> 100 m) to allow the parents to recover from the short disturbance and to habituate to the running camera. After 30 min, we walked up to the nestbox, walked back to the recording device, started the video recording and left the nest environment (sequence 1). 30 min later, we came back, interrupted and restarted the video recording for another 30 min and left again (sequence 2). The approach to the nestbox was part of another experiment, in which we wanted to investigate potential effects of a short human disturbance at the nestbox. Parents resumed feeding slightly earlier during sequence 2 compared to sequence 1 (linear-mixed effects model with log-transformed feeding latency as response variable, and sequence, year and sex fitted as fixed effects and random intercepts for brood identity and individual fitted within brood: χ^2=5.77, p=0.02; β=-0.08, 95% CI: -0.15, 0.004) but mean number of visits of both 30 min periods did not significantly differ between sequence 1 and 2 (χ^2=0.71, p=0.40), implying that human disturbance did not greatly affect parental behaviours. Therefore, we joined both recording periods for all later analyses, resulting in a total recording time of one hour per nest. The following variables of parental provisioning were retrieved from the videos: (i) individual feeding rate per hour (ii) average size of the load fed to the nestlings scored relative to the adult's bill volume (e.g. 1=volume of bill, 2=twice the volume) (iii) total biomass of the prey brought to the nest within one hour (iv) prey type summarized into the following categories: caterpillars (including sawfly larvae), other insects (mainly comprised of Diptera), spiders, white lump (presumably pulp of beechnuts) and unidentified prey items. While parental provisioning rate is a commonly used measure of parental effort it assumes that feeding rate reflects the amount of food brought to the nest. Yet, many studies

investigating parental provisioning effort in more detail have demonstrated that birds do not only show variation in feeding rate but also in the size of the prey items fed to the nestlings (Wright et al. 1998; Naef-Daenzer & Keller 1999; Grieco 2001; Mägi et al. 2009). Thus, only feeding rate in combination with load size can give a reliable estimate of the amount of food that is delivered to the nestlings. Moreover, prey type might be another important factor of parental provisioning behaviour as there is evidence that prey items can differ in their nutritional and energetic value and that parental foraging strategies involve changes in the type or range of prey items fed to the young (Royama 1966; Tinbergen 1981; Wright & Cuthill 1989, 1990; Wright et al. 1998; Grieco 2001).

We further scored begging levels of individual chicks for every parental feeding visit, at the time at which parents entered the nestbox and let go of the nestbox hole to jump down into the nest cup. We did this by estimating how high each chick stretched relative to its own height, on a scale from 0 to 10 (e.g. 0 = no begging/ resting, 10 = 100% of body height, chick fully stretches body and legs) (adapted from Kilner 1995; Wright et al. 2002). Begging posture has been shown to be a good indicator of chick need (Cotton et al. 1996; Kilner & Johnstone 1997). The correct scoring of begging levels required extensive training of observers (n=7). Observers were trained until within- and between-observer correlations were greater than 0.80 (average between-observer correlation: 0.91, range: 0.84-0.97; average within-observer correlation: 0.96, range: 0.91-0.99), which took about 4 weeks. For later analyses, we used the average chick begging level of a brood.

Statistical analyses

Year and sex differences and effects of brood size manipulation

We run linear models with the average day temperatures as response variable and year fitted as fixed effect for the months of April, May and June separately, to explore

whether weather conditions differed between years. Average day temperatures were derived from a weather station from the Bavarian regional office for agriculture, located in the centre of the study area. We used linear mixed-effect models (LMMs) with year fitted as fixed effect and random intercepts for plot to investigate whether measures of reproductive success, i.e. clutch size, hatchling number, brood size day 12, fledgling number and fledgling mass, differed between years. We ran LMMs with year and sex fitted as fixed effect and random intercepts for plot , brood identity and individual identity to test for year and sex differences of parental behaviours (feeding rate per chick, load size per chick, exploratory tendency, proportion of caterpillars included in the diet). We used feeding rate per chick as responses variables instead of overall values, enabling us to control for effects of brood size. The proportion of caterpillars was arcsine-transformed, resulting in normally distributed residuals.

We ran LMMs with the brood size manipulation (BSM) category and year and their interaction fitted as fixed effects and random intercepts for plot and brood ID to investigate whether measures of reproductive success differed between BSM categories prior and after the manipulation and whether these differences were year dependent. For models with significant year × brood size manipulation group interactions, we also ran models separately for both years to derive year-specific estimates.

Path analysis

We applied path analysis to investigate how proxies for short-term fitness (fledgling number and mass) were related to parental investment (provisioning rate and load size) and brood size at day 12 (i.e. when nestling were 12 days old). We further investigate the effects of brood size at day 1 (number of hatchlings) and BSM on brood size at day 12 and the effect of chick begging level on parental provisioning behaviour (path model A, illustrated in Figure 1). As our interest was in investigating year-specific path models (see Introduction), we ran this model for both years

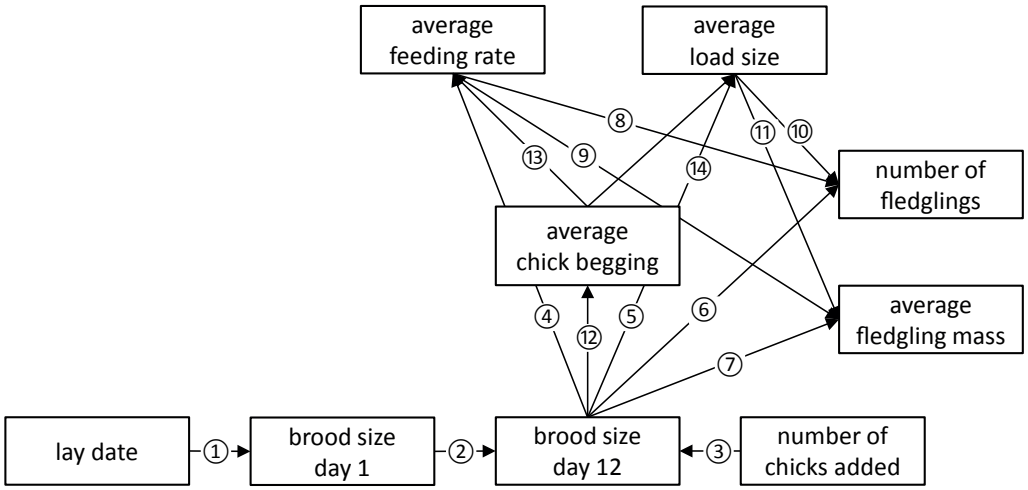


Figure 1. Hypothesized path model A. Arrows indicate the direction of hypothesized causal links. Path numbers are given in circles. Feeding rate, average chick begging and load size represent average values for a nest.

separately. As a second step, we additionally included exploratory behaviour of both sexes as a predictor variable. This path model allowed us to infer how proxies for short-term fitness were directly versus indirectly related to this behavioural trait, male and female provisioning behaviour and initial brood size (path model B, illustrated in Figure 2). To simplify the analysis, we only took those variables from path model A forward that were directly or indirectly linked to reproductive success. Previous studies have shown that selection on behavioural traits can differ across years and sexes (Dingemanse & Réale 2013). Therefore, to similarly investigate year and sex differences for all hypothesized pathways, we ran this analysis separately for each year and each sex, resulting in 4 different path models. Before running a focal path model, we first derived the variances and covariances between all predictor and response variables of the respective hypothesized path model by fitting them all as response variables into a multivariate model. We run the multivariate model within a Bayesian framework with Markov Chain Monte Carlo (MCMC) methods (MCMCglmm package: Hadfield 2010). This method enabled us to take forward the uncertainty around the variance-covariance matrix

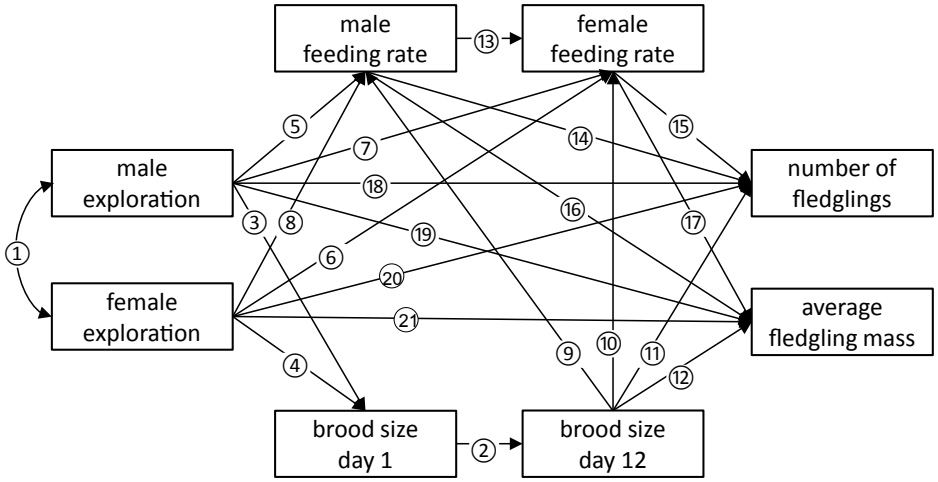


Figure 2. Hypothesized path model B. One-headed arrows indicate the direction of hypothesized causal links. Double-headed curved arrows indicate simple hypothesized correlations. Path numbers are given in circles. We also run all path models with the arrow pointing in the opposite direction.

when running the path model. We did this by running each of the specified path models once for each of the 1000 variance-covariance matrices derived from the multivariate model and then calculating the most likely path coefficient value and its associated 95% credible interval for every specified path (for details see Mutzel et al. 2013). Credible intervals not including zero indicate statistical significance in a frequentist's perspective. For intervals only slightly overlapping zero, we calculated the likelihood of an estimate being positive or negative, thus giving a value comparable with a value of P (Teplitsky et al. 2011). The strength of path analysis is that it allows calculating partial correlation coefficients between two variables while controlling for effects of all other variables in the model. This makes it a powerful tool for disentangling direct effects between two variables (e.g. variable A affects variable B), from indirect effects caused by another correlated variable (e.g. variable C directly affects B and is correlated with variable A and B). We did not have a clear prediction for the direction of the path between male and female feeding rate (path 13, Figure 2). Therefore we also ran all path models with the path linking female with male feeding rate. This did not modify any results, indicating

that this link was not caused by one sex consistently affecting the behaviour of the other sex. As all paths in the path model were hypothesized *a priori*, we present the results for the full model only, which also includes paths not supported by the model (Kontiainen et al. 2009; Mutzel et al. 2013).

Selection analysis

We applied phenotypic selection analysis to investigate how overall selection acted on male and female exploratory behaviour and parental feeding rates (in terms of reproductive success). The path analysis has the shortcoming that it cannot control for potential interaction effects between parental behaviour, reproductive success and BSM, thereby assuming that the pathways linking exploration with fitness are similar for all BSM groups. Thus, to test for brood size-specific heterogeneous selection, we applied phenotypic selection analyses to explore specifically whether the direct pathways linking exploratory behaviour and provisioning rates with reproductive success differed between the BSM groups. As selection pressures on exploratory behaviour are known to vary between years and between sexes (Dingemanse et al. 2004), we ran this analysis separately for each sex-year combination. Each fitness component was transformed into relative fitness by dividing by mean fitness of the respective data subset. Behavioural traits were standardized within year and sex to facilitate comparisons among selection gradients (Arnold & Wade 1984). We used simple linear regressions to calculate linear selection gradients, which are estimates of the direct force of selection on a given trait, while controlling for effects of other traits included in the same model (Lande & Arnold 1983). Relative fledgling number and relative fledgling mass, respectively, were fitted as response variables, while exploratory behaviour, feeding rate and BSM, and their interactions (exploratory behaviour × BSM and feeding rate × BSM), were included as fixed effects to investigate whether selection on those behaviours was a function of brood size manipulation category. BSM was fitted as a factor with three levels (reduced,

control, enlarged). Including both behaviours in the same model controls for effects of the respective other behaviour on fitness, ensuring that the reported effects of one behaviour are not due to indirect effects of the other behaviour. To test whether selection pressures significantly differed between sexes and years, we run similar models but including data of both years and sexes combined. We further fitted year and sex as additional fixed effects fitted as 4-way (and all lower-term) interactions with exploratory behaviour and BSM and feeding rate and BSM, respectively.

RESULTS

Year and sex differences in behaviour and reproduction

Average day temperatures differed significantly between years for the months of April ($F_{1,58}$=6.66, p=0.01) and May ($F_{1,60}$=11.30, p=0.001), but not June ($F_{1,58}$=0.002, p=0.96). In 2011, temperatures were on average 2.5 degrees higher in April and 3.1 degrees higher in May compared to 2010 (Figure 3). There was no difference between years in clutch size (χ^2=0.81, df=1, p=0.37) and the number of hatchlings (χ^2=2.03, df=1, p=0.15; Figure 4a), implying that initial brood sizes prior to BSM were not responsible for causing any year differences in reproductive output. Brood sizes at day 12 were, in contrast, significantly larger in 2011 compared to 2010 (χ^2=11.34, df=1, β=1.65, p<0.0001), indicating that nestling mortality was higher in 2010 than in 2011. In 2011, fledging success was also significantly higher (χ^2=36.54, df=1, β=2.94, p<0.0001; Figure 4b) and fledglings were of better condition (heavier) (χ^2=35.88, df=1, β=2.19, p<0.0001; Figure 4c) compared to 2010. Feeding rates per chick were significantly lower in 2011 (χ^2=11.13, df=1, β=-0.68, p<0.001; Figure 2d), but mean load sizes brought to the nest were significantly higher (χ^2=69.1, df=1, β=0.51, p<0.0001; Figure 4e) than in 2010, resulting in a similar amount of biomass delivered to each nestling in both years (χ^2=0.83, df=1, p=0.36; Figure 4f). As fledglings were nevertheless heavier in 2011, the energetic value of the prey

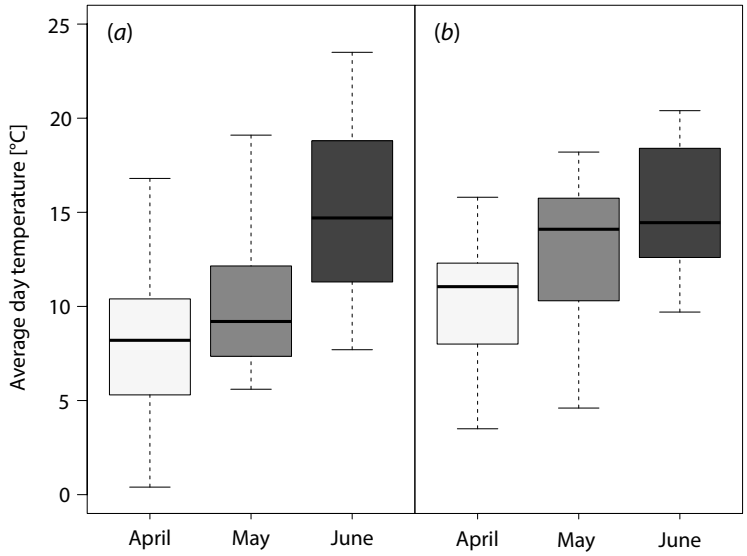


Figure 3. Average day temperatures in April, May and June for (*a*) 2010 and (*b*) 2011. Box plots show the medians, interquartile ranges and 95% CIs.

items was probably higher in 2011 and/or the nestlings needed less energy for self-maintenance because of the higher temperatures in this year. Indeed, we found that the proportion of total biomass that consisted of caterpillars, known to be a high-quality food (Royama 1970; van Balen 1973; Mägi et al. 2009), fed to the nestlings was significantly higher in 2011 (χ^2=152.04, *df*=1, β=0.73, p<0.0001; Figure 4g), whereas in 2010 the proportion of other prey types, such as other insects (mainly Diptera) and the pulp of beech nuts was also relatively high (Appendix Table A1). Nestlings were also begging significantly more in 2010 compared to 2011 (χ^2=5.99, *df*=1, β=-0.67, p=0.01; Figure 4h), implying that nestlings were generally more hungry. Altogether, these patterns imply that in 2011 conditions for breeding were better for the great tits, resulting in significantly higher reproductive success than in 2010.

Interestingly, we also found a sex difference with males feeding a significantly larger proportion of caterpillars than females (χ^2=7.98, *df*=1, β=0.12, p=0.005; Appendix Figure 1). Exploratory behaviour of adults during the breeding phase

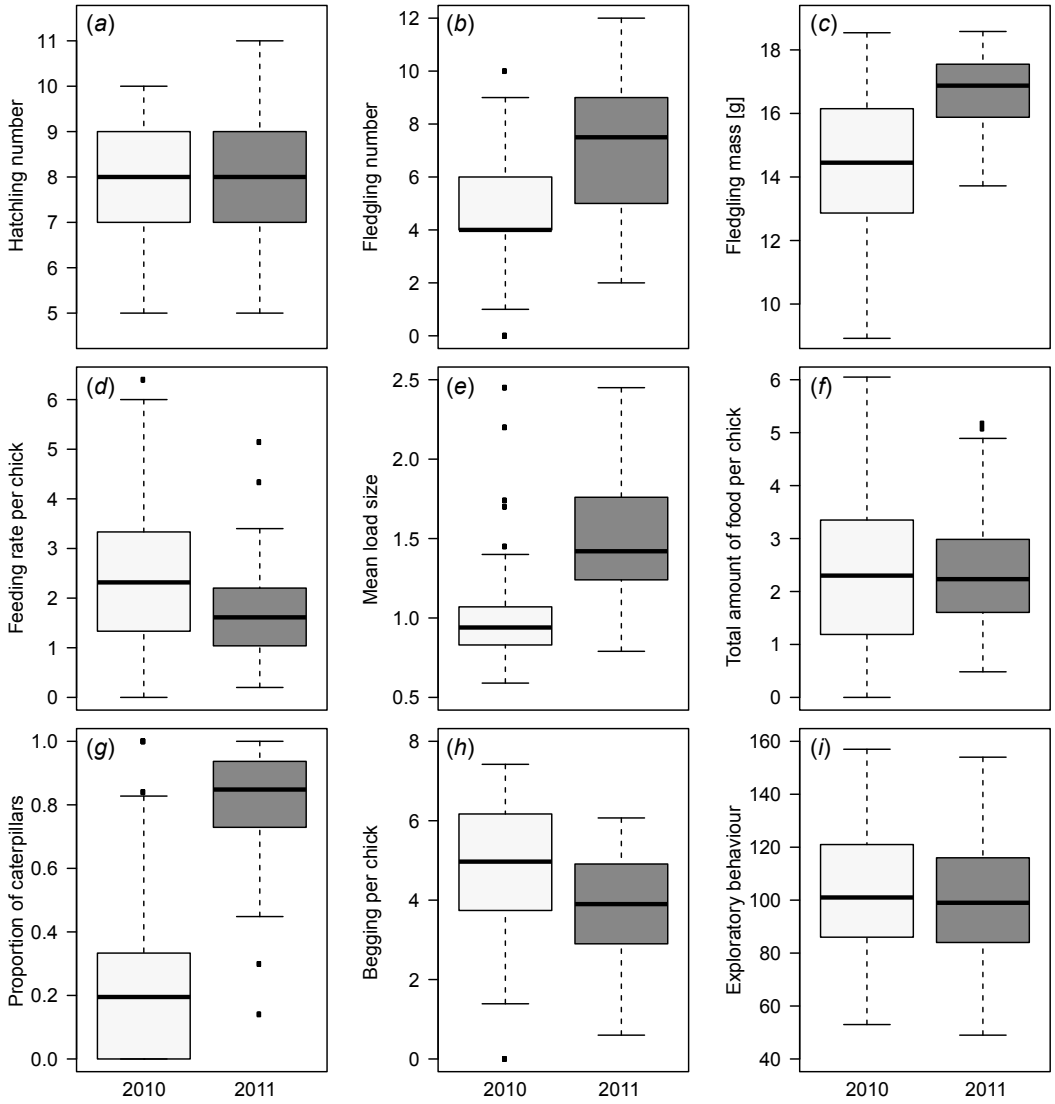


Figure 4. Year comparisons for measures of reproductive success, nestling begging levels and parental behaviours. (*a*) hatchling number (*b*) fledgling number (*c*) fledgling mass (*d*) feeding rates per chick (*e*) mean load size (*f*) total amount of food delivered per chick (*g*) proportion of caterpillars (*h*) begging level per chick and (*i*) parental exploratory behaviour. Light grey boxes give values for 2011 and dark grey boxes for 2011. Box plots show the medians, interquartile ranges, 95% CIs and outliers.

did not differ between years (χ^2=1.31, *df*=1, *p*=0.25; Figure 4i), but females had a higher exploratory tendency (i.e. were more active) compared to males in both years (χ^2=10.35, *df*=1, β=13.93, *p*<0.0001; Appendix Figure 1).

Effects of brood size manipulation on reproductive success

BSM categories did not differ from each other prior to the manipulation: neither clutch size (χ^2=1.37, *df*=2, *p*=0.50) nor the number of hatchlings (χ^2=0.51, *df*=2, *p*=0.78) differed significantly between groups. Our study also included nests that were not manipulated. We ran the same analysis with control treatment and unmanipulated nests split into two groups (resulting into 4 groups), to be able to exclude that these nests differed from the manipulated ones due to some non-random assignment to treatment versus non-treatment. Even though the results indicate that non-manipulated nests tended to differ from the other groups (χ^2=6.48, *df*=3, *p*=0.09), this effect was not very strong and was only present in the first year, where the sample size for this group was very low (*n*=5). Therefore, to simplify further analyses and to increase sample sizes, we subsequently joined control and non-manipulated broods. As expected, brood sizes at day 12 significantly differed between groups (χ^2=169.05, *df*=2, *p*<0.0001) and between years after the brood size manipulation (interaction between year and treatment group: χ^2=9.78, *df*=2, *p*=0.008) with enlarged broods in 2011 having more nestlings at day 12 than in 2010 (β=1.57, *p*=0.01). BSM also had a significant effect on the number of fledglings in 2011 (χ^2=92.28, *df*=2, *p*<0.0001) but not in 2010 (χ^2=2.79, *df*=2, *p*=0.25) (Figure 5a,b). In 2011, reduced broods had a lower (β=-2.75, *p*<0.0001) and enlarged broods a higher number of fledglings (β=1.82, *p*<0.001) compared to control broods with the strength of this effect being similar for both groups but in opposite directions (Figure 5b). In other words, the more nestlings a focal parental pair received in 2011, the more fledglings they produced. Fledgling mass also differed between BSM groups (χ^2=51.75, *p*<0.0001), and this effect did not differ between years (interaction treatment × year: χ^2=2.91, *p*=0.23). Enlarged broods were significantly lighter (β=-1.73, *p*<0.001), whereas reduced broods tended to be heavier (β=0.86, *p*<0.06) compared to control broods (Figure 5c,d).

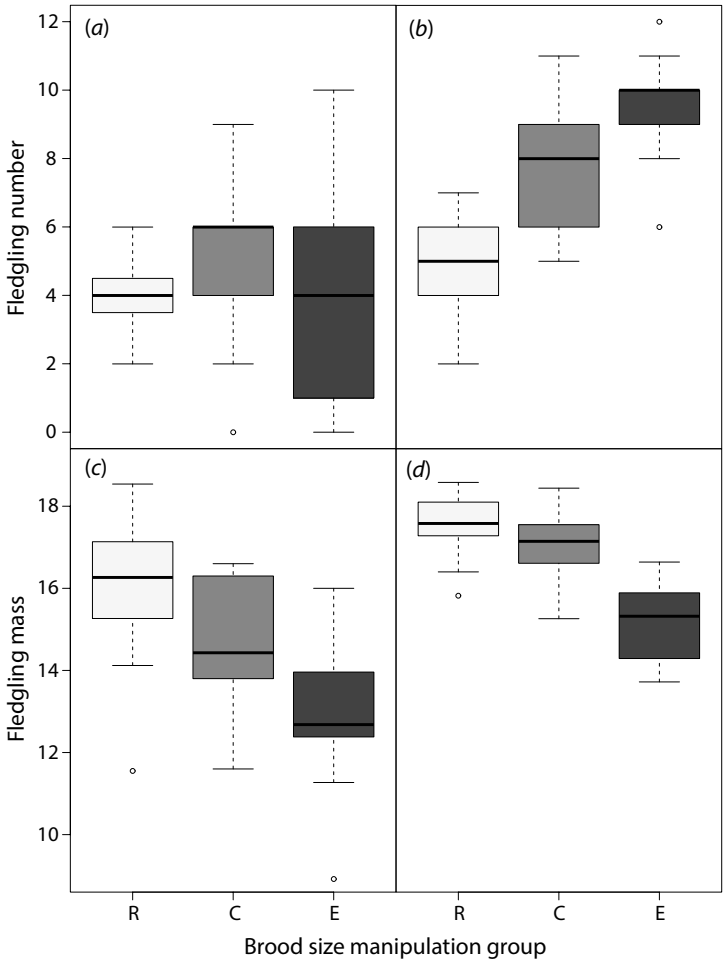


Figure 5. Effect of brood size manipulation on fledgling number and fledgling mass for (*a*) fledgling number in 2010 and (*b*) fledgling number in 2011 (*c*) fledgling mass in 2010 and (*d*) fledgling mass in 2011. Box plots show the medians, interquartile ranges, 95% CIs and outliers.

Parental effort, lay date and reproductive success

In both years, the path models A (Figure 1) supported a positive link between female lay date and the number of hatchlings, with this link being stronger in 2010 (Figure 6a,b; Table 1, path 1). To investigate whether this finding was due to early laying females having smaller clutches or having a lower hatching success, we replaced brood size with clutch size and rerun the models. While these models also gave moderate support for a positive link between lay date and clutch size in 2010, this link disappeared in 2011. This finding implies that females that initiated egg laying

Table 1. Estimated partial regression coefficients for path models A for data in 2010 and 2011. Bold numbers indicate path coefficients that are strongly supported by the model (95% credible intervals CI not overlapping zero). Italic numbers indicate path coefficients that have some support from the model (CI slightly overlapping zero but with $p < 0.05$).

		2010			2011		
path number	hypothesized link	N	path coef.	95% CI	N	path coef.	95% CI
1	lay date -> bs day 1	40	**0.50**	0.18, 0.68	46	*0.23*	-0.05, 0.51
2	bs day 1 -> bs day 12	40	**0.26**	0.03, 0.49	46	**0.47**	0.30, 0.64
3	no. chicks added -> bs day 12	40	**0.55**	0.34, 0.75	46	**0.77**	0.64, 0.92
4	bs day 12 -> feed rate	40	**0.37**	0.10, 0.63	46	**0.79**	0.58, 0.93
5	bs day 12 -> load size	40	0.12	-0.26, 0.34	46	-0.05	-0.38, 0.31
6	bs day12 -> fledgling no.	40	**0.36**	0.08, 0.60	46	**1.00**	0.89, 1.10
7	bs day12 -> fledgling mass	40	**-0.31**	-0.63, -0.05	46	**-0.53**	-0.91, -0.26
8	feed rate -> fledgling no.	40	0.27	-0.04, 0.53	46	-0.02	-0.21, 0.07
9	feed rate -> fledgling mass	40	*0.32*	-0.01, 0.61	46	-0.10	-0.36, 0.34
10	load size -> fledgling no.	40	0.03	-0.26, 0.28	46	-0.02	-0.10, 0.08
11	load size -> fledgling mass	40	0.06	-0.25, 0.31	46	0.01	-0.23, 0.23
12	bs day12 -> begging	40	0.08	-0.20, 0.40	43	**0.60**	0.32, 0.74
13	begging -> feed rate	40	-0.22	-0.34, 0.24	43	-0.01	-0.26, 0.20
14	begging -> load size	40	-0.01	-0.31, 0.33	43	-0.01	-0.42, 0.29
compound path							
A	bs day 12 -> feed rate -> fledgling mass		*0.09*	-0.03, 0.30		-0.07	-0.26, 0.28

earlier in 2010 had smaller clutches and fewer hatchlings compared to females that initiated clutches later in the season. In 2011, however, the low hatchling number of early laying females was most likely caused by a lower hatching success of early broods. Brood size at day 12 was positively affected by the number of hatchlings as well as by the BSM in both years, with both effects being stronger in 2011 (Figure 6a,b; Table 1, paths 2 and 3). This means that the larger the brood size right after hatching and the more chicks were added, the more chicks were in the nest at day 12. Both path models further revealed strong support for a positive link between

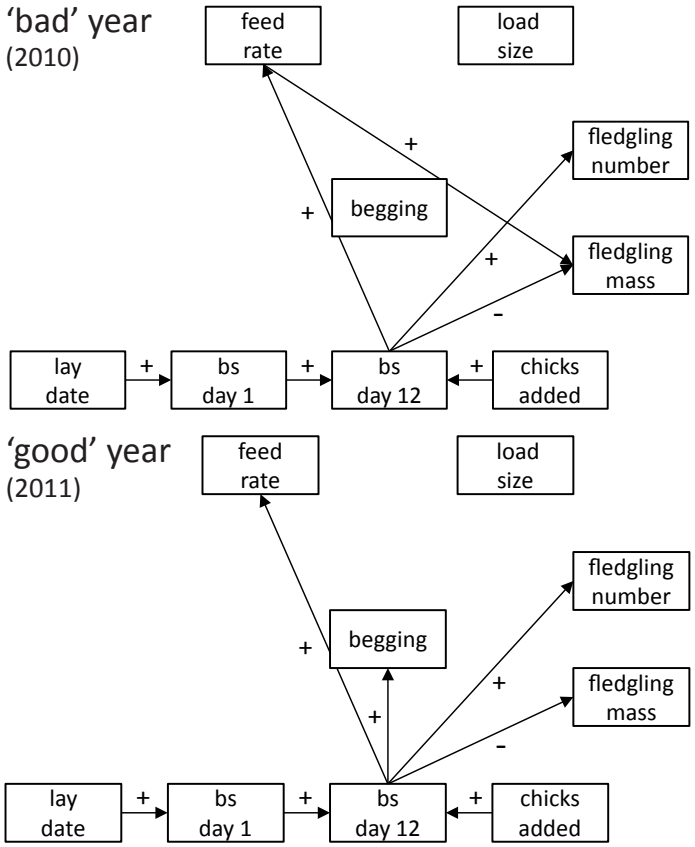


Figure 6. Supported paths in models for a bad (2010) and a good year (2011) Only paths with considerable statistical support are shown. Black arrows indicate strong support (credible intervals not overlapping zero), dashed black lines indicate some support (credible intervals overlapping zero, but with $p < 0.05$)

brood size at day 12 and parental feeding rates, but the number of chicks in the nest at day 12 did not affect load size (Figure 6a,b; Table 1, paths 4 and 5). This implies that parents with larger broods fed their chicks more often, but did not bring prey items of different size compared to parents of smaller broods. In 2010, there was no support for a link between brood size and the average chick begging level, whereas in 2011 chicks from larger broods begged more intensively than chicks that had fewer siblings (Figure 6a,b; Table 1, path 12). Chick begging level, on the other hand, did not affect parental effort in any year (Figure 6a b; Table 1, paths 13 and 14), implying that brood size played a more important role in determining parental feeding rates.

The path models B (Figure 2) revealed that parental feeding rates were directly affected by the current brood size with the exception of female feeding rates in 2010 (Figure 7a,b; Table 2, paths 9 and 10). As expected, larger broods were fed at higher rates than broods with a lower number of chicks. In 2010 only, the model also supported a link between male feeding rate and fledgling mass (Figure 7a; Table 2, path 16), but not fledging success (Figure 7a,b; Table 2, path 8), implying that in this year, fledgling condition also depended on male parental effort with males feeding their chicks at a relatively high rate for a given brood size, fledging heavier chicks.

There was also strong support for a direct link between brood size and fledgling number and mass in both years (Figure 7a,b; Table 2, paths 6 and 7). Larger broods fledged more chicks that were on average lighter than fledglings from smaller broods, indicating that parents traded-off investment in quantity versus quality of fledglings. The link between brood size and fledgling number was much stronger in 2011 compared to 2010 with nearly all nestlings surviving until fledging in 2011.

In 2010, but not in 2011, male feeding rate was directly linked to female feeding rate (Figure 7a,b; Table 2, path 13), implying that in this year males that fed their chicks at a high rate were paired to females with high feeding rates.

Exploratory behaviour and reproductive success

We did not find any evidence for a link between male or female exploratory behaviour and parental feeding rates in either year (Figure 7a,b; Table 2, paths 3 and 4), thereby failing to confirm the hypothesis that parental provisioning behaviour mediates the link between exploratory behaviour and reproductive success. However, in 2011, we found support for an alternative indirect pathway via which female exploratory behaviour affected reproductive success, mediated by brood size. Female exploratory behaviour was directly linked to the number of

Table 2. Estimated partial regression coefficients for path models B for data in 2010 and 2011. Bold numbers indicate path coefficients that are strongly supported by the model (95% credible intervals CI not overlapping zero). Italic numbers indicate path coefficients that have some support from the model (CI slightly overlapping zero but with $p < 0.05$).

		2010			2011		
path number	hypothesized link	N	path coef.	95% CI	N	path coef.	95% CI
1	♂ exploration -> ♀ exploration	34	0.19	-0.13, 0.49	44	0.26	-0.08, 0.48
2	bs day 1 -> bs day 12	41	**0.35**	0.02, 0.59	46	**0.46**	0.18, 0.64
3	♂ exploration -> bs day1	41	-0.18	-0.46, 0.12	45	-0.03	-0.28, 0.27
4	♀ exploration -> bs day 1	34	0.13	-0.17, 0.44	44	**0.34**	0.03, 0.58
5	♂ exploration -> ♂ feed rate	41	0.13	-0.20, 0.34	45	-0.09	-0.30, 0.17
6	♀ exploration -> ♀ feed rate	34	0.19	-0.14, 0.43	44	-0.09	-0.26, 0.20
7	♂ exploration -> ♀ feed rate	41	-0.11	-0.33, 0.17	45	0.04	-0.26, 0.17
8	♀ exploration -> ♂ feed rate	34	-0.01	-0.32, 0.25	44	-0.02	-0.20, 0.27
9	bs day 12 -> ♂ feed rate	41	**0.47**	0.15, 0.68	46	**0.56**	0.33, 0.73
10	bs day 12 -> ♀ feed rate	41	0.14	-0.22, 0.33	46	**0.77**	0.50, 0.91
11	bs day 12 -> fledgling no.	41	**0.42**	0.16, 0.67	46	**1.00**	0.87, 1.08
12	bs day 12 -> fledgling mass	39	**-0.32**	-0.63, -0.05	46	**-0.75**	-0.94, -0.36
13	♂ feed rate -> ♀ feed rate	41	**0.56**	0.27, 0.76	46	-0.11	-0.38, 0.15
14	♂ feed rate -> fledgling no.	41	0.23	-0.11, 0.48	46	-0.03	-0.14, 0.06
15	♀ feed rate -> fledgling no.	41	0.14	-0.19, 0.40	46	-0.07	-0.13, 0.08
16	♂ feed rate -> fledgling mass	39	**0.50**	0.17, 0.82	46	0.06	-0.24, 0.27
17	♀ feed rate -> fledgling mass	39	-0.10	-0.45, 0.17	46	-0.04	-0.27, 0.29
18	♂ exploration -> fledgling no.	41	-0.15	-0.35, 0.14	45	**0.08**	0.001, 0.17
19	♂ exploration -> fledgling mass	39	-0.03	-0.29, 0.25	45	0.001	-0.20, 0.24
20	♀ exploration -> fledgling no.	34	**0.34**	0.11, 0.63	44	0.01	-0.07, 0.10
21	♀ exploration -> fledgling	34	**0.36**	-0.001, 0.62	44	-0.04	-0.25, 0.18

hatchlings (Figure 7a,b; Table 2, paths 5-8), with fast-exploring females having more hatchlings compared to slow explorers. When hatchling number was replaced with clutch size, the model also revealed a positive, but much weaker, link (path coefficient exploration -> clutch size: 0.24, 95%CI: -0.08-0.52), suggesting that fast-exploring females laid slightly larger clutches and additionally had a higher hatching success compared to slow-exploring individuals. At the same time,

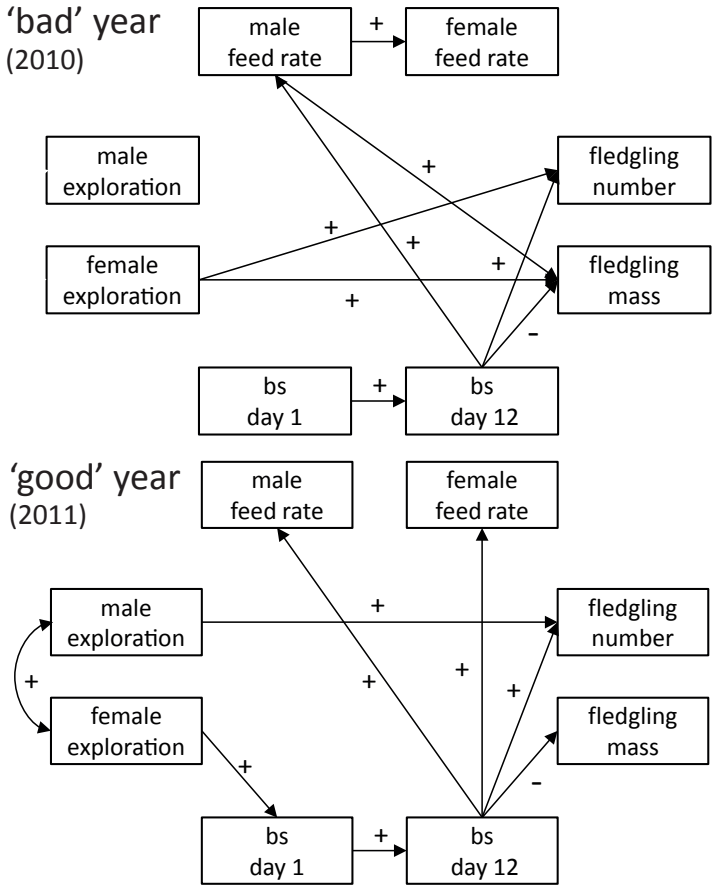


Figure 7. Supported paths in models for a bad (2010) and a good year (2011). Only paths with considerable statistical support are shown. Black arrows indicate strong support (credible intervals not overlapping zero), dotted black lines indicate moderate support (credible intervals overlapping zero, but with $p < 0.10$)

hatchling number positively affected brood size at day 12, whereas brood size positively affected fledgling number and negatively affected fledgling mass (Figure 7a,b; Table 2, paths 2, 11 and 12). This resulted in fast-exploring females having more fledglings with a lower weight, whereas slow explorers fledged fewer chicks in better condition (Table 2, compound paths A and B). These findings suggest that females with opposite exploration types resolved the trade-off between fledgling number and condition in different ways. Interestingly, in 2010, female exploratory behaviour also affected reproductive success, but via an unknown pathway (Figure 7a; Table 2, paths 20 and 21). Fast-exploring females fledged more offspring which

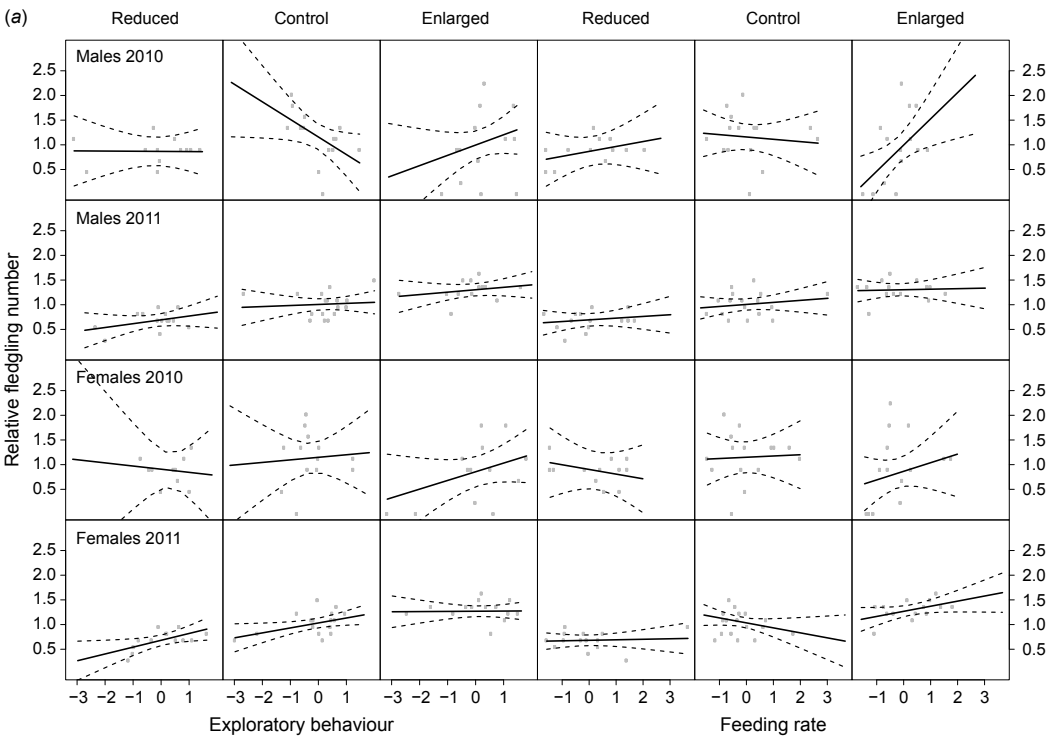
were in better conditions, indicating that these females were doing better than slow-exploring ones in this year.

Even though models of both years did not support any indirect pathways linking male exploratory behaviour with reproductive success, there was a weak but significant direct effect of male exploration on fledgling production in 2011 (Figure 7b; Table 2, path 18), with fast-exploring males managing to fledge a relatively high number of their chicks.

The path model also gave moderate, but non-significant (p=0.09), evidence for a link between male and female exploratory behaviour in 2011 but not in 2010. This suggests that fast-exploring males tended to be paired to fast-exploring females, whereas slow-exploring males tended to have slow exploring partners. Alternatively, the habitat of a pair might have affected male and female behaviour simultaneously, resulting in relatively similar exploration scores.

Selection analysis

While selection analysis revealed an overall positive selection gradient for male feeding rates in 2010 and treatment-specific selection in 2011, there was no selection acting on females provisioning rates. This implies that male but not female provisioning behaviour directly affected reproductive success and that this effect was strongest under poor environmental conditions. In contrast, in females, fast-exploratory tendency was selected for in both years, while selection gradients for male exploratory behaviour differed between the BSM categories and were only present in 2010. This implies that female exploratory behaviour predicted reproductive success and that this effect was no mediated via female feeding rates.


(a)
Reduced
Control
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Control
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Males 2010
Males 2011
Females 2010
Females 2011
Relative fledgling number
Exploratory behaviour
Feeding rate

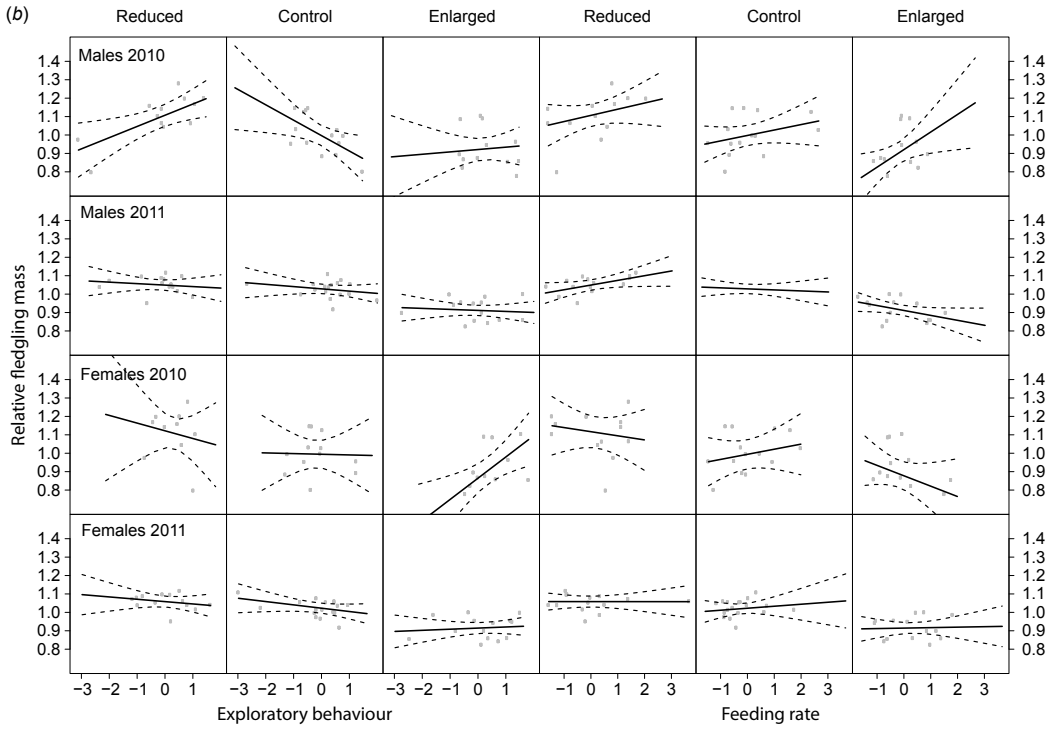
(b)
Reduced
Control
Enlarged
Reduced
Control
Enlarged
Males 2010
Males 2011
Females 2010
Females 2011
Relative fledgling mass
Exploratory behaviour
Feeding rate

Figure 8 . Parental exploratory behaviour and provisioning rate (standardized within year and sex) against (*a*) relative fledgling number and (*b*) relative fledgling mass for the different brood size manipulation groups. Fitted value lines (with 95% CIs) were derived from 4 linear models (split by year and sex) with the two-way interactions between exploratory behaviour and brood size manipulation and provisioning rate and brood size manipulation group fitted as fixed effects. Grey dots represent raw data.

Selection gradients for male behaviours

In males, there was evidence for directional selection on male feeding rate in 2010, which was independent of the treatment group, with males feeding at a higher rate having heavier fledglings than males that fed their nestling less often (selection gradient: β±SE: 0.05±0.01; Table 3b ; Figure 8b). We could also reveal treatment-specific selection pressures acting on male exploratory behaviour and male feeding rate in 2010 (Table 3a). Selection favoured slow-exploratory tendency within the control treatment group, with slow-exploring males producing more and heavier nestlings compared to fast explorers. Yet, when confronted with a manipulated brood size, slow explorers did relatively badly compared to fast explorers that coped equally well over the entire brood size range (Table 4a,b; Figure 8a,b). Within the reduced treatment group, fast exploratory tendency was even selected for with fast-exploring males having fledglings in better condition than slow exploring ones (Table 4b; Figure 8b).While there was no selection acting on feeding rate within the control treatment group, selection strongly favoured males with high feeding rates when confronted with an enlarged brood size with frequently feeding males producing more fledglings (Table 4a, Figure 8a).

In 2011, selection on male feeding rate became apparent only when males were confronted with manipulated broods sizes (Table 3b). Within the reduced broods group, selection tended to favour high feeding rates with males feeding their chicks at high rates having fledglings in better condition, whereas there was negative

Table 3. Linear regressions on relative fledgling number and relative fledgling mass with brood size manipulation treatment (treat: factor with 3 levels) fitted as interaction with exploratory behaviour and feeding rate for (*a*) fledgling number (*b*) fledgling mass. Sample sizes were lower for fledgling mass due to missing mass values for broods that died between day 12 (recording of provisioning behaviour) and day 14 (weighing of nestlings).

		2010		2011	
		$F_{eff.df,err.df}$	*p*	$F_{eff.df,err.df}$	*p*
(*a*)					
Males	treat	$1.41_{2,32}$	0.26	$27.35_{2,36}$	**<0.0001**
	exploration	$0.01_{1,32}$	0.95	$1.92_{1,36}$	0.17
	feeding rate	$1.89_{1,32}$	0.18	$0.92^{1,36}$	0.34
	treat × exploration	$3.03_{2,32}$	**0.06**	$0.22_{2,36}$	0.80
	treat × feeding rate	$3.21_{2,32}$	**0.05**	$0.07_{2,36}$	0.93
Females	treat	$1.32_{2,27}$	0.29	$35.30_{2,35}$	**<0.0001**
	exploration	$3.68_{1,27}$	**0.07**	$5.35_{1,35}$	**0.03**
	feeding rate	$0.40_{1,27}$	0.53	$0.39_{1,35}$	0.54
	treat × exploration	$0.45_{2,27}$	0.65	$2.28_{2,35}$	0.12
	treat × feeding rate	$0.61_{2,27}$	0.55	$2.73_{2,35}$	**0.08**
(*b*)					
Males	treat	$11.98_{2,30}$	**<0.001**	$30.18_{2,36}$	**<0.0001**
	exploration	$2.38_{1,30}$	0.13	$0.68_{1,36}$	0.42
	feeding rate	$9.24_{1,30}$	**<0.01**	$0.05_{1,36}$	0.82
	treat × exploration	$5.80_{2,30}$	**<0.01**	$0.15_{2,36}$	0.86
	treat × feeding rate	$1.06_{2,30}$	0.36	$3.79_{2,36}$	**0.03**
Females	treat	$7.62_{2,25}$	**<0.01**	$27.67_{2,35}$	**<0.0001**
	exploration	$2.58_{1,25}$	0.12	$0.93_{1,35}$	0.34
	feeding rate	$0.02_{1,25}$	0.88	$0.07_{1,35}$	0.80
	treat × exploration	$2.29_{2,25}$	0.12	$0.95_{2,35}$	0.40
	treat × feeding rate	$1.21_{2,25}$	0.32	$0.13_{2,35}$	0.88

selection on male feeding rate within enlarged broods with males with low feeding rates having heavier fledglings (Table 4b, Figure 8b).

Table 4. Standardized linear selection gradients on exploratory behaviour and feeding rate in wild great tits in 2010 and 2011 split by brood size manipulation treatments for (*a*) fledgling number and (*b*) fledgling mass. Selection gradients were derived from linear regression with relative fitness (fledgling number or fledgling mass) fitted as response variable and exploratory behaviour, feeding rate and brood size manipulation treatment, and their interactions, fitted as fixed effects. Models were run separately for each of the fitness estimate and each year and sex. Significant or close to significant selection gradients are given in bold. The hash and stars indicate the degree of significance (# $p<0.1$, * $p<0.05$, ** $p<0.01$).

		Exploratory behaviour			Feeding rate		
		reduced	control	enlarged	reduced	control	enlarged
	year	*β*±SE	*β*±SE	*β*±SE	*β*±SE	*β*±SE	*β*±SE
(*a*)							
Males	2010	-3.49×10^{-2} ±0.11	**-0.36±0.17***	0.21±0.16	0.10±0.13	-0.05±0.12	**0.53±0.20***
	2011	0.08±0.07	0.02±0.06	0.05±0.06	0.03±0.06	0.04±0.05	0.01±0.06
Females	2010	-0.06±0.29	0.05±0.19	0.18±0.13	-0.09±0.16	0.03±0.15	0.17±0.18
	2011	**0.14±0.06***	**0.10±0.05***	3.46×10^{-2} ±0.05	0.01±0.04	-0.10±0.07	**0.10±0.06#**
(*b*)							
Males	2010	**0.06±0.02***	**-0.08±0.04***	0.01±0.03	0.03±0.03	0.03±0.02	0.10±0.04
	2011	-0.01±0.01	-0.01±0.01	-0.01±0.01	**0.03±0.01#**	-0.01±0.01	**-0.03±0.01***
Females	2010	-0.04±0.07	-0.01±0.05	**0.12±0.04****	-0.02±0.04	0.03±0.04	-0.06±0.04
	2011	1.27×10^{-2} ±1.71×10^{-2}	-0.02±0.01	0.01±0.01	-8.34×10^{-5} ±1.10×10^{-2}	0.01±0.02	2.56×10^{-2} ±0.02

Selection gradients for female behaviours

In females, we found evidence for an overall positive selection effect on exploratory tendency which was independent of brood size treatment. This effect was mediated by fledgling production (Table 3a), with fast-exploring females having a higher number of fledglings compared to slow-exploring ones in 2011 (β±SE: 0.06±0.03; Figure 8a) and tending do to so in 2010 (β±SE: 0.13±0.09; Figure 8a). In 2011, there was also a non-significant treatment-specific selection effect on female feeding rate (Table 3a). While female feeding rate did not affect fledgling production in the control or reduced brood size groups, females with high feeding rates tended to fledge more offspring compared to slow-feeding females within the enlarged treatment group (Table 4a; Figure 8a).

Sex and year differences

To investigate whether treatment-specific selection effects were year- or sex-specific, we ran models with the complete dataset and including 4-way (and all lower-term) interactions between treatment, sex, year and exploratory behaviour or feeding rate, respectively. For fledgling production, the 4-way interactions with exploratory behaviour as well as with feeding rate were non-significant, implying that treatment-specific effects were not depending on a year-sex interaction. Yet, we found a significant treatment-specific effect on exploratory behaviour that differed across years (treatment × year × exploration: $F_{2,130}$=3.21, p=0.04, Appendix Table A2), implying that there was heterogeneous selection across years. For fledgling mass, we found a significant 4-way interaction for exploratory behaviour (treatment × exploration × year × sex: $F_{2,126}$=3.33, p=0.04, Appendix Table A2) and a close to significant 4-way interaction for feeding rate (treatment × feeding rate × year × sex: $F_{2,126}$=5.98, p=0.05, Appendix Table A2), implying that treatment-specific selection effects on both behaviours differed indeed across years and sexes, even though these effects were rather weak.

DISCUSSION

This study provides evidence that exploratory behaviour covaries with reproductive success. Yet, selection on behavioural phenotypes differed between brood size manipulation groups with fast explorers being better in raising a high number of chicks than slow-exploring parents. Moreover, selection on exploratory behaviour also differed across years, with selection being stronger in a year with low food availability. We further show that parental feeding rate did not play a role in mediating links between behaviour and reproductive success. Yet, we found some evidence that behavioural types differed in another aspect of parental provisioning behaviour, namely the type of prey delivered to nestlings.

Year differences in reproductive success

Reproductive success differed drastically between the two years of our study. In 2010, overall nestling survival rate was extremely low with only 65% of the hatchlings surviving till fledging, whereas survival rate in the second year of the study was about 20% higher. These year differences in nestling survival were also reflected in the outcome of the brood size manipulation. Brood sizes of the different treatment groups differed significantly in 2011, with parents being able to fledge nearly all nestlings that they received in their nest. In contrast, brood size manipulation did not affect the number of fledglings in 2010, implying that parents were not able to increase food delivery sufficiently to satisfy the increased energy demand of enlarged broods. This is further underlined by the finding that fledglings in this year were on average about two grams lighter compared to the second breeding season. The low breeding success in 2010 was most likely caused by the exceptionally cold spring, where average day temperature in April and May were about 3°C lower than in 2011. This is because low spring temperatures often result in a mismatch between nestling peak and the peak of caterpillar abundance (Kluijver 1951; Perrins

1979, 1991; van Noordwijk et al. 1995), the preferred food for great tits nestlings (Royama 1970; van Balen 1973; Gosler 1993; Mägi et al. 2009). Even though we do not have data on prey availability in our study population, we found that the proportion of caterpillars in the total diet provisioned to nestlings was more than 50% lower in 2010 than in 2011 (27% in 2010 versus 80% in 2011). As previous studies have shown that the prey types brought to the nest are highly dependent on their availability in the immediate nest environment (Naef-Daenzer & Keller 1999; Naef-Daenzer et al. 2000), this strongly suggests that caterpillar abundance in 2010 was indeed very low.

Parental effort and reproductive success

In our study population, when looking at both years separately, only parental provisioning rates, but not load sizes, were a reliable estimate of the amount of food delivered to nestlings. This is an interesting finding, as previous studies on great tits suggested that the size of prey items can vary considerably, mainly depending on the size of the prey available to the birds, (Naef-Daenzer & Keller 1999; Mägi et al. 2009) and we therefore had expected to find an effect of load size on the delivered biomass. Possibly, the variation in prey size within year was not large enough to be able to cause a detectable effect or to be captured by our measurement method. Yet, when comparing load sizes between years, we were able to detect highly significant differences in the size of prey items which also translated into the total biomass delivered to nestlings. Even though prey items brought to the nest were much smaller in 2010 compared to 2011, parents compensated for small loads in the first year by increasing their feeding rate, ultimately feeding each nestling with a similar amount of food. Yet, fledglings were still of much better condition in the second year. This might indicate that caterpillars, the main nestling food in 2011, offer a higher energetic and nutritional value compared to other prey types (cf. Perrins 1991; Naef-Daenzer & Keller 1999; Wilkin et al. 2009), such as Diptera, the main

nestling food in 2010. This is consistent with previous studies, showing a decline in feeding frequency with an increase in the proportion of caterpillars in the diet and thus suggesting that great tits try to compensate for small and poor- quality prey items by visiting the nest more often in caterpillar poor years (Mägi et al. 2009; Wilkin et al. 2009).

We found that parental effort, in terms of provisioning rates, positively affected reproductive success in the bad year, but this was only true for male feeding rates. Males, that fed their nestlings at a higher rate, fledged offspring in better condition. This finding was supported by the results of the selection analysis which revealed a positive directional selection effect on male feeding rate via fledgling mass. In addition, selection also favoured high male feeding rates via an increased fledgling production, but only within the enlarged brood size manipulation group. This now raises the question of why selection in this year acted on male but not on female feeding rate. This could possibly be explained by between-individual variation in feeding rates being much lower in females compared to males. However, rather the opposite was the case with females differing more in how often they fed each chick (Appendix Figure A1). Another explanation could be that males differed from females in other aspects of provisioning behaviour which were closely related to feeding rate, thereby impacting offspring condition. Indeed, we found that males brought slightly larger prey items and a higher proportion of caterpillars. Males further showed higher between-individual variation in these aspects than females, resulting in males bringing more and higher-quality food to the nest at each visit, thereby having a higher impact on reproductive success.

In 2011, we found no evidence for a link between parental feeding rates and reproductive success. This was probably because there was heterogeneous selection acting on the different treatment groups, whereby cancelling out any overall selection effect. While selection favoured high male feeding rates within the reduced treatment group through frequently feeding males having heavier

fledglings, this effect levelled out for the controls and acted in the opposite direction within enlarged broods. In contrast, selection acted on female feeding rates only within enlarged broods with females with high feeding rates having higher fledging success. The finding that selection in this year only acted in a treatment-specific way and differed between sexes, strongly suggests that males and females responded to the brood size manipulation in different ways. Whereas all females seemed to be able to provide their offspring with a sufficient amount of food when confronted with reduced or control brood sizes, some females did better than others, when they had to feed enlarged broods. This could be due to some females being more constraint or less willing to increase their feeding rates enough to satisfy chick need. Indeed, it is commonly assumed that in great tits, females are more likely to be energetically constrained during nestling feeding than males, as females have to invest more energy in the early breeding phase (i.e. in egg production and incubation) (Visser & Lessells 2001).The heterogeneous selection acting on male feeding rate is an interesting finding, as high feeding rates should always be selected for under the assumption that feeding rate is a good estimate for the total amount of energy delivered to the nest. Possibly, males responded to the brood size manipulation by changing load sizes or prey types rather than adjusting feeding rates, with males bringing bigger prey types and/or more caterpillars with increasing brood sizes. For instance, previous studies on great tits have demonstrated a negative correlation between the amount of food brought to the nest and feeding frequency (Kluijver 1950; van Balen 1973). Indeed, we found some evidence, even though non-significant, that males feeding enlarged broods brought larger prey items than males with reduced or control broods, whereas this was not the case in females (Appendix Figure A2). This suggests that the unexpected observed negative selection against high feeding rates might have rather been the result of direct positive selection acting on prey size which was coupled with reduced feeding frequency.

But how did the year differences in selection pressures come about? The strongest link between feeding rate and reproductive success was found in the first year, with a directional selection effect generally favouring high male feeding rates. This is not surprising, given that food availability in this year was relatively low, making it difficult for the parents to provide their offspring with a sufficient amount of food. This resulted in a relatively high between-individual variation in fledgling condition on which selection could act on. In contrast, in 2011, food availability was high enough that all parents managed to provide their nestlings with a sufficient amount of food, resulting in a low variance in fledgling mass. As the variance in fitness places an upper bound on the force of selection that can act on a phenotypic character (Arnold & Wade 1984), this could explain the only weak selection on parental feeding rates in this year. Such year- and sex-dependent effects of parental provisioning behaviour on fledgling condition, might also explain, why previous studies on great tits and blue tits investigating the link between provisioning effort (in terms of total biomass delivered to nestlings) and reproductive success produced rather inconsistent results (e.g. no link: Nour et al. 1998; link: Naef-Daenzer & Keller 1999).

Behavioural pathways linking exploratory behaviour and reproductive success

Exploratory behaviour and feeding rate

Our study did not provide any evidence for a link between exploratory behaviour and provisioning effort, thereby failing to confirm our hypothesis that parental provisioning behaviour plays a role in mediating the link between parental exploratory behaviour and reproductive success. This is in contrast to a recent study on blue tits, *Cyanistes caeruleus*, showing that fast-exploring females fed their nestlings at a higher rate, thereby positively affecting fledgling production (Mutzel et al. 2013). This study, however, only investigated natural brood sizes, whereas

brood sizes of the present study were manipulated. It is possible that we would have detected a similar link in great tits when looking at natural brood sizes only. This would be the case if links between exploratory behaviour and provisioning rates were caused by exploratory behaviour being closely related to other factors directly or indirectly impacting feeding rates, such as brood size or habitat quality (over its effect on clutch size). Unfortunately, we did not have enough control broods to test this hypothesis, but further studies investigating in more detail how brood size manipulation affects the links between personality traits and provisioning behaviour could shed more light on this question.

Female exploratory behaviour and brood size

Even though we could not reveal a link between exploratory behaviour and parental provisioning rates, female exploratory behaviour still affected reproductive success via an alternative mechanistic pathway but only in one year. In 2011, fast-exploring females had larger brood sizes compared to slow explorers, resulting in a higher number of fledglings that were in worse condition. This indirect pathway most likely also caused the overall positive selection acting on female exploratory tendency in this year mediated via fledgling production. Similarly, in blue tits, female exploratory behaviour has been shown to be linked with fledgling production mediated by fast-exploring females initiating egg laying earlier in season and having larger brood sizes (Mutzel et al. 2013). In contrast, the larger brood sizes of fast-exploring great tits were independent of lay date and were most likely mediated by a combination of fast explorers laying larger clutches and having a higher hatching success compared to slow-exploring individuals. This suggests that fast-exploring females were able and/or willing to invest more time and energy in the current reproduction. This is because the costs of egg production (Monaghan & Nager 1997; Visser & Lessells 2001) as well as of nestling feeding increases with increasing brood size. The higher hatching success of fast-exploring females might

indicate that they maintained higher incubation temperatures compared to slow explorers, which is also assumed to be associated with a higher energy expenditure (Webb 1987). Such a high investment in the current reproduction is consistent with theoretical life-history trade-off models that predict that aggressive, fast-exploring and risk-taking individuals have moderate future fitness expectations and should therefore invest more in the current brood (Wolf et al. 2007; Biro & Stamps 2008). Another not mutually exclusive explanation could be that fast-exploring females bred in high-quality habitats and therefore were in better body condition at the onset of the breeding season compared to slow explorers. This is supported by the finding that fast-exploring females tended to be paired to fast-exploring males that have previously been shown to breed in high-quality territories (Dingemanse & De Goede 2004; Both et al. 2005). A good breeding habitat, with a high abundance of high-quality nestling food, might explain at the same time why fast-exploring males produced slightly more fledglings. But why did we not find a similar link between female exploratory behaviour and the number of hatchlings in 2010? Possibly, environmental conditions in this year were so bad that even fast-exploring females could not afford to invest much in egg production and incubation and/or were not able to build up extra energy resources early on in the breeding season (Nager et al. 1997).

Exploratory behaviour and reproductive success

We found a strong link between female exploratory behaviour and reproductive success in 2010, with fast-exploring females fledging more offspring in better condition, but via a pathway not considered in our model. This was also confirmed by the selection analysis, revealing positive, but non-significant, selection pressures on female exploratory tendency mediated via fledging production. This implies that fast-exploring females coped relatively well with the adverse environmental conditions of the first year. In males, on the other hand, the path model did not reveal

any direct or indirect link between exploratory behaviour and reproductive success. Yet, selection still acted on male exploratory tendency but in a treatment-specific way, thereby potentially obscuring links within the path model. Selection strongly favoured slow exploratory tendency under natural environmental conditions (i.e. control brood sizes) by slow-exploring males having more and heavier fledglings. In contrast, fast-exploratory tendency was selected for in males with a reduced brood size with fast-exploring males having slightly heavier fledglings. This implies that slow explorers were doing relatively well when confronted with natural brood sizes, but relatively bad when their brood size was reduced.

What mechanisms could be responsible for causing the detected links between parental exploratory behaviour and reproductive success? As only about half of the nestlings of a brood were genetic offspring of the parents (due to the swapping of nestlings between broods during brood size manipulations) it is unlikely that these links were caused by genetic or maternal effects. Behavioural phenotypes further had similar brood sizes right after hatching, implying that the variation in reproductive success was caused by some sort of mechanisms improving nestling survival. These mechanisms, however, must be unrelated to the amount of food delivered to nestlings as male and female provisioning rates and load sizes were not linked with exploratory tendency. Yet, as already stated above, not only the amount of food fed to nestlings, but also its energetic and nutritional value are important for nestling survival and condition. Thus, one possible explanation for the strong link between exploratory tendency and reproductive success could be that certain exploratory types provided their nestling with a higher proportion of high-quality prey types, such as caterpillars. We investigated whether exploratory tendency was related to the proportion of caterpillars biomass brought to the nest to test the hypothesis that exploratory behaviour was linked to the quality of the prey delivered to nestlings. Indeed, we found some weak evidence that slow explorers tended to bring more caterpillars than fast explorers (β=-0.002, p=0.09). This might

be explained by slow individuals exploring their environment more thoroughly (Verbeek et al. 1996; Drent & Marchetti 1999), thereby more efficiently detecting cryptic and patchily distributed preys types such as caterpillars (Naef-Daenzer & Keller 1999; Grieco 2001). Alternative mechanisms causing differential selection on behavioural types could be driven by individuals differing in how much time and energy they are willing to invest in the current brood. This is underlined by current life-history trade-off theories suggest that high investment in current reproduction comes at the cost of reduced survival. These models predict that fast, aggressive and risk-taking individuals have a lower survival probability and therefore should invest more in the current reproduction, whereas slow and risk-averse individuals are generally more likely to survive until the next breeding season and should therefore only show moderate investment in the current reproduction (Wolf et al. 2007; Biro & Stamps 2008). Under this theory, fast explorers are expected to show increased investment in behaviours that enhance reproductive success.

But how could this explain the different selection patterns acting on male and female exploratory behaviour? Whereas females can increase their reproductive output only by investing more in their own brood, males can enhance their reproductive success also by acquiring extra-pair offspring. This might result in fast-exploring females showing higher investment in aspects of parental care other than provisioning rate. For instance, they might spend more time and energy in brooding the young, defending their offspring more vigorously against predators or searching for high-quality food thereby enhancing nestling survival. Fast-exploring males, on the other hand, might try to enhance their reproductive success by acquiring extra-pair offspring and/or defending their territory more aggressively against conspecific competitors. In contrast, slow-exploring males might save their time and energy resources for offspring provisioning. Especially in bad years, when food is rare and hard to find, females might not be able to compensate for a low male provisioning effort (Bart & Tornes 1989), resulting in reproductive success strongly depending

on a joint parental effort. Indeed we found that, in the first year, individuals matched their feeding rates to their partner's feeding effort, whereas this was not the case in the second year when food availability was relatively high.

This now raises the question of why slow male exploratory tendency was only selected for under natural environmental conditions, i.e. control brood sizes, whereas fast-exploratory behaviour was selected for in males with a reduced brood size. As slow explorers are commonly assumed to be more sensitive towards changes in their environment (Verbeek et al. 1994; Drent & Marchetti 1999), they might be more strongly affected by the brood size reduction than fast explorers. Whereas the latter might have ignored or not even noticed the manipulation, slow-exploring males might have taken note of the brood size reduction and perceived the sudden disappearance of nestlings as an indicator of a risky environment and a potential threat to the surviving nestlings (Tilgar & Kikas 2009). As this should result in a decreased expected fitness value of the remaining offspring (Curio 1987; Tilgar & Kikas 2009), slow-exploring males are expected to reduce their investment in these broods, ultimately producing fledglings of lower condition. This hypothesis is further supported by the finding that, in females with reduced brood sizes, selection also acted against slow-exploratory tendency in the second year.

Conclusions

This study provides evidence for natural selection acting on exploratory behaviour in a wild population of great tits. Yet, selection acting on behavioural phenotypes differed between brood size manipulation groups, emphasizing the importance of experimental studies when investigating personality-fitness relationships. Moreover, we demonstrate that selection pressures differed across years and sexes, suggesting that heterogeneous selection might be a possible mechanism for maintaining behavioural variation in great tits. This work further revealed that parental feeding rates were not part of a mechanistic pathway mediating links between behaviour

and reproductive success. In contrast, we found some evidence for behavioural phenotypes differing in clutch size and in the quality of the prey delivered to nestlings. Further studies are now needed to investigate if other habitat parameters, such as variation in predation risk, population density or mating strategies, might be involved in mediating the link between exploratory behaviour and reproductive success.

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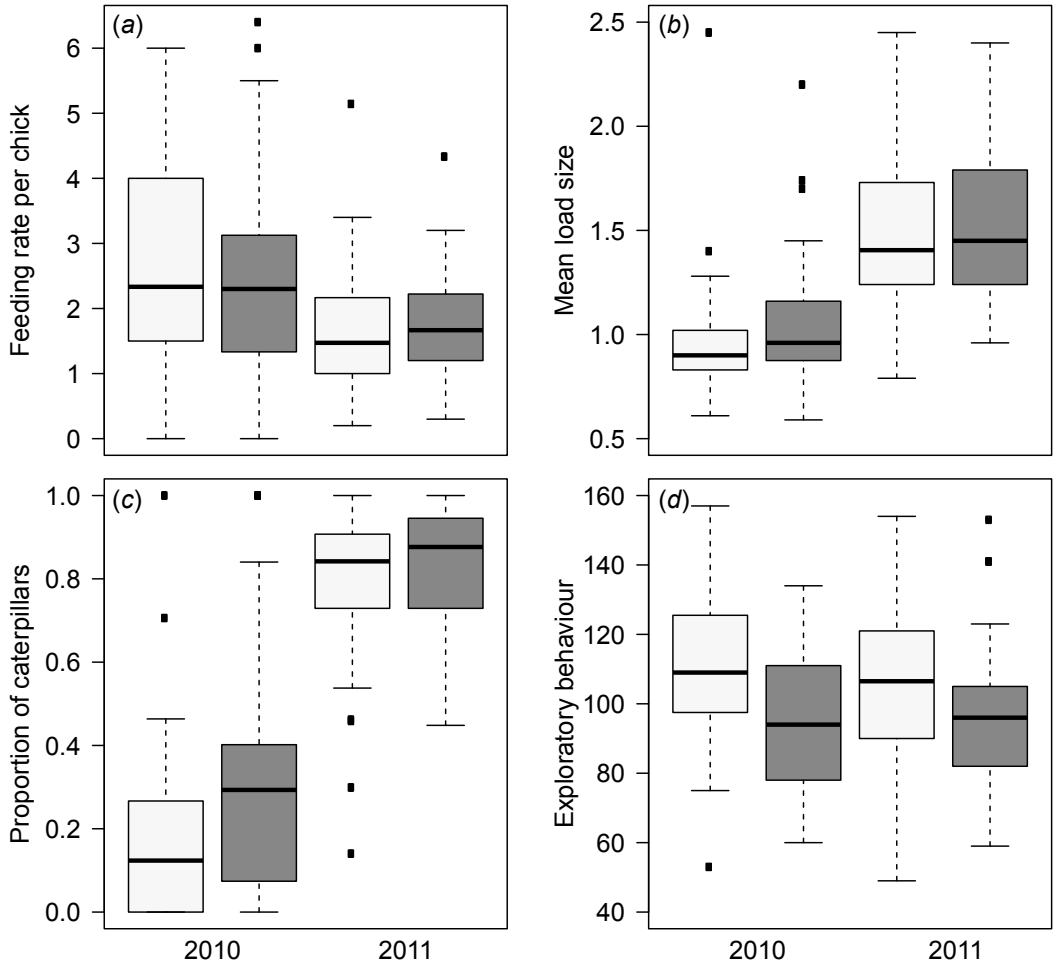
APPENDIX

Appendix Table A1. Proportion of total biomass delivered to nestlings split by prey type and year

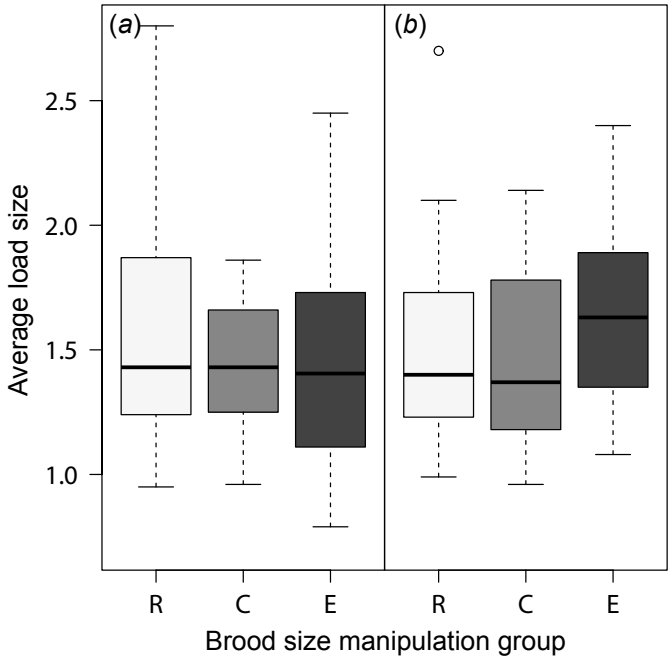
	Caterpillar	Other insects	Beech nut	Spider	Unidentified
2010	27.2	38.3	18.5	3.3	12.7
2011	80.0	7.9	0.6	2.6	8.9

Appendix Table A2. Linear regressions on relative fledgling number and relative fledgling mass with brood size manipulation treatment (treat: factor with 3 levels) fitted as 4-way and all lower-term interactions with year, sex and exploratory behaviour or feeding rate, respectively.

	Fledgling number		Fledgling mass	
	$F_{eff.df,\ err.df}$	*p*	$F_{eff.df,\ err.df}$	*p*
treat	$12.72_{2,130}$	**<0.0001**	$53.36_{2,126}$	**<0.0001**
year	$0.39_{1,130}$	0.53	$0.01_{1,126}$	0.92
sex	$0.08_{1,130}$	0.77	$0.01_{1,126}$	0.99
exploration	$4.58_{1,130}$	**0.03**	$2.02_{1,126}$	0.16
feeding rate	$4.69_{1,130}$	**0.03**	$4.38_{1,126}$	**0.04**
treat × year	$10.54_{2,130}$	**<0.0001**	$2.63_{2,126}$	**0.08**
treat × sex	$0.22_{2,130}$	0.80	$0.05_{2,126}$	0.95
year × sex	$0.21_{1,130}$	0.65	$0.01_{1,126}$	0.92
treat × exploration	$1.71_{2,130}$	0.18	$5.80_{2,126}$	**<0.01**
year × exploration	$0.01_{1,130}$	0.96	$4.64_{1,126}$	**0.03**
sex × exploration	$2.00_{1,130}$	0.16	$0.42_{1,126}$	0.52
treat × feeding rate	$3.17_{2,130}$	**0.04**	$0.46_{2,126}$	0.63
year × feeding rate	$1.24_{1,130}$	0.27	$3.36_{1,126}$	**0.07**
sex × feeding rate	$0.55_{1,130}$	0.46	$1.66_{1,126}$	0.20
treat × year × sex	$0.01_{2,130}$	0.99	$0.14_{2,126}$	0.87
treat × year × exploration	$3.21_{2,130}$	**0.04**	$4.28_{2,126}$	**0.02**
treat × sex × exploration	$1.33_{2,130}$	0.27	$1.75_{2,126}$	0.18
year × sex × exploration	$0.57_{1,130}$	0.45	$1.76_{1,126}$	0.19
treat × year × feeding rate	$1.87_{2,130}$	0.16	$0.33_{2,126}$	0.72
treat × sex × feeding rate	$0.12_{2,130}$	0.89	$0.86_{2,126}$	0.43
year × sex × feeding rate	$0.72_{1,130}$	0.40	$4.76_{1,126}$	**0.03**
treat × year × sex × exploration	$0.82_{2,130}$	0.44	$3.33_{2,126}$	**0.04**
treat × year × sex × feeding rate	$2.17_{2,130}$	0.12	$2.98_{2,126}$	**0.05**



Appendix Figure A1. Sex comparisons for both years for different aspects of parental provisioning behaviour and exploratory behaviour. (*a*) feeding rate per chick (*b*) mean load size (*c*) proportion of caterpillars (*d*) exploratory behaviour. Light grey boxes give values for females and dark grey boxes for males. Values for 2010 are given on the left side of each panel, values for 2011 on the right side. Box plots show medians, interquartile ranges, 95% CIs and outliers.



Appendix Figure A2. Average load sizes delivered to nestlings in 2011 for (*a*) females and (*b*) males for the 3 different brood size manipulation categories. R = reduces, C = control, E = enlarged

General Discussion

The aim of this thesis was to shed light on the mechanistic pathways linking behavioural variation with fitness. I address this question both from a proximate and ultimate perspective. In chapter 1, I investigate the underlying physiological basis of personality differences. Chapters 2, 3 and 4 explore via which mechanistic pathways selection might act on personality. I provide observational and experimental evidence that testosterone is not part of a proximate mechanism causing individual variation in exploratory behaviour and activity in male house sparrows (chapter 1). Using two different study systems, I further show that exploratory behaviour (chapters 3 and 4) and aggressiveness (chapter 3) covary with reproductive success, implying that behavioural phenotypes are subject to natural selection. Moreover, I provide evidence that parental care behaviours, such as offspring provisioning and nest defense, play an important role in mediating the link between personality and reproductive success (chapters 2, 3 and 4).

Proximate mechanisms underpinning behavioural variation

Hormones typically influence many aspects of behaviour and physiology, making them suitable candidates for mediating variation in behaviour (Ketterson & Nolan 1992). Yet, to be able to explain consistent variation in behaviour would require hormone levels to consistently differ between individuals. Such individual differences could come about, if hormone levels represent an inherently stable state, i.e. that inherent between-individual differences in hormone levels cause variation in behaviour. However, hormone levels are often considered as labile states, as they are typically affected by many different factors, including the behaviour of other

individuals or an individual's behaviour itself (Koolhaas et al. 2010). This scenario could explain consistent individual differences only when initial differences in these labile states are stabilized by positive feedback mechanisms (Dingemanse & Wolf 2010). For instance, an individual with a relatively high initial testosterone level might behave more aggressively towards a conspecific territory intruder. The aggressive interaction might positively affect an individual's testosterone level, and therefore promote further aggressive behaviour consequently resulting in relatively stable high levels of hormone.

In chapter 1, I provide observational and experimental evidence that individual differences in circulating plasma testosterone levels do not underpin variation in exploratory behaviour and activity (Mutzel et al. 2011). We further show that plasma testosterone levels in different seasons were correlated, indicating relatively long-term repeatability of testosterone. Nevertheless, we still know very little about long-term stability of individual variation in testosterone levels, and how such consistent differences might come about, i.e. whether they are inherently stable or the result from positive feedback mechanisms.

Our study showed that levels of testosterone were not correlated with exploratory behaviour and activity (Mutzel et al. 2011). Yet, testosterone might still be involved in creating individual variation in other behavioural traits. For instance, it is commonly assumed that testosterone affects the expression of aggressive behaviours across a wide array of taxa (Wingfield et al. 1987; Ketterson & Nolan 1999), while, at the same time, it typically decreases levels of parental care (Wingfield et al. 1987; Ketterson & Nolan 1999; Duckworth 2006a). Such a testosterone-based mechanism could also proximately explain the findings of chapter 3, where I show that aggressive males have lower feeding rates compared to less aggressive individuals. In contrast, male exploratory behaviour, which has been shown to be unrelated to testosterone levels (chapter 1; Mutzel et al. 2011), was not linked with levels of parental care (Mutzel et al. 2013a). It remains to be tested, whether aggressive male blue tits

indeed have higher testosterone levels, which also causally affect their investment in offspring provisioning.

Maintenance of personality variation

A key question in the field of animal personality is why different types of individuals coexist within the same population. Classis explanations for the maintenance of behavioural variation include heterogeneous forms of selection, such a temporal and spatial fluctuation in selection pressures, sexually antagonistic selection and frequency dependent selection, and other types of selection such as correlational selection and life-history trade-offs. Here, I address some of the suggested mechanisms.

Spatio-temporal fluctuating selection

Spatio-temporally fluctuation in selection occurs when environmental conditions (e.g. availability of food or other limited resources) differ across time or space. In chapter 4, I found for great tits that weather conditions across the two years of the study differed drastically, resulting in pronounced differences in the amount and type of food available to great tits for feeding their nestlings (cf. Kluijver 1951; Perrins 1979). In females, fast-exploratory tendency was only selected for in the year where food availability was extremely low. This suggests that fast-explorers had a selective advantage over slow-exploring individuals only under adverse environmental conditions. However, we do not have replication to test whether selection on exploratory tendency was indeed directly related to food resources during a breeding season, or rather caused by another factor differing between years (e.g. predation risk) that we did not consider in our study. Possibly, when food is abundant, all behavioural phenotypes might manage equally well to provide their nestlings with a sufficient amount of food, resulting in a relatively low variance in fledgling condition. As the variance in fitness places an upper bound on the force

of selection that can act on a phenotypic character (Arnold & Wade 1984), this should result in relatively weak selection acting on behaviour in good years. In contrast, the study on blue tits (chapter 3) was carried out in one single season, thus not allowing us to evaluate whether the reported selection pressures were general versus year-specific.

Sexually antagonistic selection

Sexually antagonistic selection is expected when males and females differ in their optimal values for the same trait, while partly the same underlying genes are expressed in both sexes, resulting in strong positive cross-sex genetic correlation (Cox & Calsbeek 2009). As males and females are likely to experience different (social) environments and have to face different challenges (e.g. energetic and nutritional requirements), optimal behaviour is also likely sex-specific (cf. Sprenger et al. 2012). This thesis provides evidence that selection pressures acting on behavioural traits fluctuate indeed among sexes (chapters 3 and 4). In both female blue tits and great tits, fast exploratory tendency was selected for with fast explorers feeding their nestling at higher rates (chapter 3) and producing larger clutches (chapters 3 and 4), whereas both studies did not reveal any clear selection pattern acting on exploratory behaviour in males (chapters 3 and 4; Mutzel et al. 2013a). This is potentially because females can increase their reproductive success only by investing more in their own brood, whereas males can enhance their reproductive success also by acquiring extra-pair offspring, which we did not take into account in our studies. Another, non-exclusive explanation might be that, in males, other behavioural traits are more important in determining reproductive success. For instance, reproductive success could be mediated via aggressiveness: aggressive males might be able to acquire high-quality territories and defending the brood more vigorously against nest predator. If, at the same time, female aggressiveness is selected against, by less aggressive females investing more in parental care, this could result in sexually

antagonistic selection on aggression. Yet, we did not investigate whether there are also cross-sex genetic correlations for these behaviours. Hence, the results of this study do only provide some support for the first part of the predictions of sexually-antagonistic selection theory. More studies are needed to shed further light on the question of whether this mechanism plays a role in maintaining behavioural variation in great tits.

Life-history trade-offs

Another mechanism by which variation in personality could be maintained, involves trade-offs between different life-history traits (Wolf et al. 2007; Biro & Stamps 2008; Réale et al. 2009). For example, a theoretical model by Wolf and coworkers (2007) proposed that aggressive, fast-exploring and risk-taking individuals should be able to acquire high-quality resources and thus to reproduce early in life. At the same time they face a higher mortality risk compared to less aggressive individuals and are therefore expected to have a relative short reproductive period. The low assets, i.e. low future fitness expectations, of aggressive males should result in a relatively risk-prone behaviour, whereas less aggressive individuals should behave in a relatively risk-averse fashion to protect these assets (Wolf & Weissing 2010). Consequently, asset protection theory predicts that aggressive and exploratory individuals should be willing to invest highly in the current brood, whereas less aggressive individuals should only show moderate investment in the current reproduction. In our study on blue tits, we show that fast-exploring females indeed seemed to invest more in their current brood by provisioning their offspring at higher rates and producing larger clutches than slow-exploring individuals, thereby increasing their reproductive success (chapter 3; Mutzel et al. 2013a). Similarly in great tits, we found that fast-exploring females had a higher reproductive success than slow explorers. Fast-exploring great tits produced larger clutches and had a higher hatching success, indicating that they were willing to invest more time and

energy in egg production and incubation (chapter 4; Webb 1987; Monaghan & Nager 1997; Visser & Lessells 2001). However, the current study did not investigate whether fast-exploring females also live less long, though this prediction has been confirmed by meta-analysis (Smith & Blumstein 2008).

Importantly, life-history models explaining consistent variation in personality are based on the assumption that individuals differ in state and that behaviour itself is state-dependent. Examples for state variables that have been suggested to underpin individual differences in behaviour are the hypothalamic-pituitary-adrenal (HPA) axis (Koolhaas et al. 1999), level of energy reserves (Rands et al. 2003) or the type of environment an individual lives in, e.g. habitat quality (Wolf & Weissing 2010). Identifying the state variables underpinning individual differences in behaviour may thus provide important insights into the evolution and maintenance of variation in personality. For example, Wolf et al. (2010) predicted that risky behaviours should differ between individuals whenever those individuals differed in future fitness expectations. This idea has been tested in a wild population of great tits by experimentally manipulating individual survival probability and thus the future fitness expectations. This study found that individuals with decreased survival probability became more risk-prone compared to bird with increased survival probability, thus confirming the predictions of the asset protection hypothesis (Nicolaus et al. 2012).

In summary, my work provides evidence that temporal fluctuating selection, sexually antagonistic selection as well as trade-offs between current and future reproduction may play a role in maintaining behavioural variation in natural populations of blue tits and great tits. These mechanisms can possibly explain why standing between-individual variation in behaviour might be preserved but it cannot, however, explain *why* individuals differ consistently in behaviour (Stamps 2007). Thus, future empirical observational and experimental studies should focus on testing the predictions of these so-called ‘state-dependent’ models, and other holistic

explanations for the phenomenon. Moreover, investigating mechanistic pathways linking behaviour with fitness might shed light on how individual differences in state could maintain variation in behaviour.

Mechanistic pathways linking behaviour with fitness

There is accumulating evidence that behavioural traits covary with proxies for fitness such as survival or reproductive success (Dingemanse & Réale 2005; Réale et al. 2007; Smith & Blumstein 2008; Dingemanse & Réale 2013), implying that behavioural phenotypes are subject to natural selection. Yet, to understand *how* natural selection acts on personality, it is necessary to investigate the mechanistic pathways linking personality with fitness. One behavioural mechanism that is a likely candidate for mediating this link involves parental care. This is because parental care directly affects reproductive success (Clutton-Brock 1991), while at the same time behavioural phenotypes have been hypothesized to differ in their parental ability (Both et al. 2005) and their levels of investment in parental care (Roulin et al. 2010).

In chapters 3 and 4 we specifically investigated if parental care is involved in mediating personality with reproductive success. While in blue tits, parental provisioning rates played a key role in mediating this link (chapter 3), there was no evidence for such a pathway in great tits (chapter 4). This implies that parental care can indeed mediate personality-specific variation in reproductive success, but that there must also be other mechanistic pathways that need to be identified.

In chapter 3, we indeed found a link between male aggressiveness and provisioning behaviour. Surprisingly, although aggressive males fed their nestlings at low rates, male aggressiveness had a positive effect on fledgling production. This was because their low feeding rates were associated with an increased female effort (Mutzel et al. 2013a). This is an intriguing finding, since parental care is costly (Clutton-Brock

1991) and both members of a pair should consequently benefit from investing less than their partner (Westneat & Sargent 1996; Houston et al. 2005). This thus raises the question of why female partners of aggressive males are willing to take a larger share of the burden of offspring provisioning compared to females paired to less aggressive individuals. This would make sense if females still benefit from being paired to aggressive males. For example, aggressive males might provide their partners with other resources, such as good genes or high-quality territories (Dingemanse & De Goede 2004; Both et al. 2005; Duckworth 2006b; Scales et al. 2013), or might invest more in other important aspects of parental care behaviour, e.g. nest defense (Duckworth 2006a; Hollander et al. 2008).

Although in blue tits both sexes typically engage in nest defence behaviour and offspring provisioning without any clear patterns of a sex-specific division of labour, there is some evidence that blue tits might divide different parental care behaviours among partners with one pair member investing more in nest defense, while the other one continues offspring provisioning (Chapter 2; Mutzel et al. 2013b). Such a division of labour could help to ensure immediate nestling survival while at the same time mitigating potential negative long-term consequences of feeding interruptions for offspring growth and condition, thus maximizing fitness. Possibly, aggressive males take a larger share of defending the brood against potential nest predators, while their mates, in return, put more effort into offspring provisioning (Chapter 3; Mutzel et al. 2013a). An exciting future avenue would be to investigate in more detail, whether division of parental care behaviours among pair members is indeed linked to personality.

In chapter 2, I further demonstrate that blue tits consistently differ in their response towards a potential predation threat to their nestlings. This raises the interesting question of whether consistent between-individual differences in this aspect of parental care could be part of a behavioural mechanism linking personality with reproductive success.

In chapters 2, 3 and 4 I provide evidence that one important aspect of parental care, namely offspring provisioning behaviour, plays an important role in mediating the link between personality and fitness. Yet, the underlying mechanisms for why behavioural phenotypes differ in provisioning behaviour remain still obscure. Such a link could be explained by behavioural phenotypes differing in their *ability* to provide this type of parental care. For instance, behavioural types might differ in their cognitive abilities or how they process information (Mathot et al. 2012; Sih & Del Giudice 2012). Such individual differences are likely to directly or indirectly, e.g. via foraging ability or territory quality (e.g. Verbeek et al. 1994; Drent & Marchetti 1999; Dingemanse & De Goede 2004; Scales et al. 2013), affect parental care capabilities. Indirect effects might also be mediated by individual variation in the ability to adequately respond to variation in nestling demand, with some types being more responsive than other, thereby increasing their reproductive success (Roulin et al. 2010). Behavioural phenotypes might also differ in their *willingness* to invest time and energy in the current brood (see above), resulting in individual differences in levels of parental care (cf. Wolf et al. 2007). Although these explanations are non-exclusive, it could explain personality-related differences in parental care even without involving other aspects such as foraging ability or territory quality. One important next step to shed more light on the underlying mechanisms linking personality with parental care is therefore to not only quantify personality, parental care behaviours and reproductive success. Instead, we should also investigate at the same time whether behavioural phenotypes differ in other aspects, e.g. in survival probability, in the type of environment they live in (e.g. habitat quality or brood size), in their responsiveness towards nestling demand or foraging behaviour. Most importantly, future studies should try to identify the underlying basic mechanisms for behavioural phenotype-specific differences in parental care or foraging ability. For instance, empirical studies should investigate how behavioural traits relate to different aspects of cognition or information processing (Mathot et al. 2012; Sih & Del Giudice 2012).

The importance of experimental studies when investigating links between personality and fitness

Our understanding of the mechanistic pathways linking personality with parental care is currently hampered by the observational nature of most studies. This is because studies typically do not take into account that parental care decisions are plastic and largely a function of breeding habitat in which the birds find themselves. Features of breeding habitats include, for example, territory quality, different aspect of social environment or brood size. When the choice of the breeding habitat depends on the behavioural phenotype of the individual, this might obscure potential links between personality and parental care. For instance, the finding that exploratory tendency is not linked with feeding rate might be explained by individuals not differing in how much they are able or willing to invest in the current brood. Alternatively, fast-exploring females are able and willing to invest more in reproduction than slow explorers, but are somehow constraint to produce their optimal clutch size (Monaghan & Nager 1997). For instance, there could be physiological constraints for laying optimal clutch sizes (Monaghan & Nager 1997; Visser & Lessells 2001). This might obscure any link between personality and parental effort, if personality types differ in these constraints. To break apart such potential personality-environment correlations, it is necessary to manipulate the breeding environment, here brood size, in order to confront different behavioural types with the entire range of environmental variation. We did this by dividing broods into three brood size manipulation categories (reduced, control, enlarged). The experimentally manipulated brood demand consequently enabled us to test which behavioural types do well versus poorly under which type of condition.

Chapters 3 and 4, indeed provide evidence for personality related differences in clutch sizes with fast-exploring females laying larger clutches, suggesting that experimental manipulations of brood sizes are warranted to help understand personality-related differences in parenting behaviour. Moreover, in chapter 4, we

show that behavioural phenotypes also differ in how they cope with (experimentally manipulated) small, control and large brood sizes. For instance, even though fast-exploring females generally had a higher reproductive success than slow explorers, this effect became most apparent when they were forced to work harder. In males, slow explorers with natural brood sizes had a higher reproductive success than fast explorers, while they were doing relatively badly, when forced to raise enlarged broods. Overall, these results suggest that fast explorers are better in raising a high number of chicks than slow-exploring parents, but that there were specific constraints hindering fast explorers to produce their optimal clutch size. These findings further suggest that measuring parental effort with unmanipulated brood sizes gives only a limited insight about the ability and willingness of the different behavioural phenotypes to invest in parental care, thus emphasizing the importance of experimental studies.

Conclusions und future directions

In this work, I identified an important proximate mechanistic pathway linking personality with fitness, by demonstrating that parental care plays an important role in mediating links between exploratory behaviour and aggressiveness with reproductive success. I further provide convincing evidence that circulating levels of plasma testosterone do not underpin variation in exploratory behaviour and activity, and are thus unlikely to be part of a proximate mechanisms linking these two behavioural traits with fitness. Future studies should try to detect other mechanisms involved in linking behaviour with fitness. Potential variables that are likely to be part of such mechanistic pathways include foraging behaviour, responsiveness towards external clues, risk-taking behaviour or mating strategies.

I further showed that selection on personality is temporal and sexually heterogeneous and that behavioural phenotypes might differ in how they trade-off current with future reproduction. Despite accumulating evidence for mechanisms maintaining

behavioural variation, we still know very little about how *consistent* variation in behaviour could be maintained. Thus, empirical studies are now needed to test the predictions and assumptions of the theoretical models for the maintenance of such repeatable variation in nature.

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Author Contributions

Chapter 1

BK, JD and AM conceived the study. AM, JD, BK and SL contributed to the design. AM developed and conducted the behavioural assays and collected the blood samples. AM and NJD analyzed the data. AM, JD and BK wrote the manuscript with input from all co-authors. The observational data of this study were collected and used for the Diploma Thesis of AM. All other data were collected afterwards and the statistical analysis and the writing of the manuscript were done within the framework of this thesis.

Chapter 2

AM, NJD and JW conceived and designed the study using data collected from a field study designed by AM with input from BK. AM, MB and FS were involved in fieldwork. AM, NJD and JW analyzed the data and wrote the manuscript. All co-authors contributed to revisions.

Chapter 3

AM and NJD conceived and designed the study with input from BK using data collected from a field study designed by AM with input from BK. AM collected the data. AM, YGA and NJD conceived the statistical approach and analyzed the data. AM and NJD wrote the manuscript with input from all co-authors.

Chapter 4

AM and NJD conceived and designed the study. AM and NJD were involved in data collection. AM and NJD performed the statistical analysis. AM and NJD wrote the manuscript with input from BK.

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Declaration

Ehrenwörtliche Versicherung

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